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Improvement of Rice Through Somaculture (Somaclonal Variation, Plant Histology, Cell Culture).

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IMPROVEMENT OF RICE THROUGH SOMACULTURE

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IMPROVEMENT OF RICE THROUGH SOMACULTURE

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural And Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Plant Pathology and Crop Physiology

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ABSTRACT

Research was conducted to develop methods for improving rice through somaclonal variation using plant tissue culture techniques. Experiments were directed toward developing an efficient culture system by which plants could be efficiently regenerated in rice somaculture. Histological studies of the process of callus formation and plant regeneration were also carried out.

Explant sources evaluated for their potential for callus induction and plant regeneration included seedling mesocotyl, root-tips, embryos from mature seeds, nodes, immature panicles, and mature panicles. Immature panicles gave the best callus induction and plant regeneration. It was determined that it was necessary to add 2,4-D to the medium to initiate callus induction. The combination of indoleacetic acid (0.5 mg/l) and benzyladenine (0.8 mg/l) or naphthaleneacetic acid (2 mg/l) and kinetin (1 mg/l) in Murashige-Skoog medium gave the best plant regeneration. It was found that 3% sucrose was optimum for both callus formation and plant regeneration. Callus induction from immature panicle explants was highest after incubation in the dark at 28°C. Plant regeneration was highest with incubation under fluorescent light for 16 hours at 28°C. More effective callus growth and plant regeneration were obtained when small calli (20-40 mg) were transferred to plant regeneration medium.

More than 5,000 plants were regenerated from several U.S. and foreign cultivars. Two hundred somaclones from the rice cultivar Labelle and one hundred from the cultivar Lemont were evaluated for

variation in morphological and agronomic characters and for yield potential in the field in 1985. An additional 358 lines from Labelle and 218 lines from Lemont were tested in the field for changes in resistance to the rice sheath blight disease. Variation was observed in the following characters: albinism, days to heading, sterility, leaf blade angle, flag leaf angle, culm angle, leaf color, leaf pubescence, leaf senescence, culm strength, resistance to rice sheath blight, panicle type, panicle exsertion, sterility, seedling height at the maximum tillering stage, mature plant height, panicle length, panicle number, panicle weight, 100-seed weight, and yield. Lemont somaclonal line 726 yielded 12% more than the Lemont cultivar.

INTRODUCTION

Tissue culture is rapidly becoming an important tool for rice breeding and genetics. Tissue culture studies with rice began in Japan in the mid 1950's. Successful somaculture and gametoculture of rice was conducted in Japan in the late 1960's. A complete review of the literature on this subject is given in Appendix 1. In spite of the early successes with rice tissue culture, many aspects of rice somaculture and gametoculture need to be investigated before techniques are available to routinely use tissue culture in rice breeding and genetics programs. In addition, aspects of rice tissue culture related to culturing of single cells and regeneration of plants from single cells must be elaborated before successful transformation of rice can be carried out. The objectives of this investigation were:

1. To develop an efficient system for regenerating rice plants through somaculture.
2. To study the morphology and histology of callus formation and plant regeneration in rice somaculture.
3. To study the effects of cultivar, nutrition, and culture conditions on somaculture of rice.
4. To study embryogenesis in cell suspension cultures of rice.
5. To study the somaclonal variation generated through somaculture of rice.

The six chapters of this dissertation are written in manuscript form for submission to scientific journals. Chapters 1,2 and 4 are written in the style of the journal PLANT SCIENCE. Chapter 3 is

written in the style of the journal PROTOPLASMA. Chapters 5 and 6 are written in the style of the journal CROP SCIENCE. Each chapter is a complete discussion of one aspect of this research and includes methods, results and discussion, and literature citation sections. A comprehensive literature review and complete details of methods used in these studies are given in the appendices.

CHAPTER I

AN EFFICIENT CULTURE SYSTEM FOR PLANT REGENERATION FROM IMMATURE PANICLES OF RICE (*Oryza sativa* L.)

ABSTRACT

Comparative studies were conducted on the effects of explant source on callus formation and plant regeneration in rice. Explant sources used in this experiment included root tips, mesocotyl, mature embryos, endosperm, nodes, and immature and mature panicles. Immature panicles were the best explant sources for callus formation and plant regeneration. An efficient culture system was established through culture of immature panicles. More than 5,000 plants were regenerated from a number of U.S. and foreign cultivars. Characteristics of callus induction and plant regeneration from immature panicles were recorded. The stage of development of immature panicles was a major factor in induction of callus formation and regeneration of plants.

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; CH, casein hydrolysate; BA, N^6-benzyladenine; MS, Murashige and Skoog medium; IAA, indolacetic acid.

Introduction

The introduction and manipulation of genetic variation are the principal means by which plant breeders realize their objectives. Generation of genetic variability through plant cell and tissue culture shows potential for use in crop improvement [1]. This has stimulated an interest by plant breeders in exploring the possibility of improving crop plants, gramineous crops in particular, using tissue culture techniques. Before somaculture can be used as a breeding tool, it is necessary to establish an efficient culture system through which callus can be quickly induced, increased, and plants rapidly regenerated.

Callus initiation and subsequent shoot formation and plant regeneration in rice have been accomplished from diverse somatic origins including root [2, 3], leaves [4], immature and mature endosperm [5, 6], leaf sheaths [7], seedlings [8], and mature embryos [9] since the first demonstration of callus formation and plant regeneration from somatic tissue of rice in 1968. Recently, the efficiency of plant regeneration has been greatly increased by the identification of embryogenic and non-embryogenic callus in tissue culture and the utilization of immature and meristematic tissue explants for the establishment of totipotent cultures [10]. It has been reported in rice that immature panicles can be used to regenerate plants by somaculture [11-13]. A historical review on plant somaculture in relation to improvement of rice is given in Appendix 1 [14]. This study compared the efficiency and reliability of a range of rice explant sources used for callus induction and subsequent plant regeneration.

Materials and Methods

The 12 rice (Oryza sativa L.) cultivars Brazos, Cr1113, Labelle, Leah, Lebonnet, Lemont, Melrose, Nato, Taipei 309, Tadukan, Tetep, and Zenith were used as explant sources.

The explants used were the mesocotyl region of seedlings germinated in the dark, 1-cm seedling root tips, culm nodes, immature panicles (1 to 6 cm long in the boot), mature panicles (over 10 cm long, green), embryos from mature seeds, and endosperm from mature seeds.

The details of preparation of explants for callus initiation are given in Appendices 4, 5, and 6 [14]. Explants were sterilized in 1.6% sodium hypochlorite solution for 30 min. and rinsed with sterile water three times. The explants were cut into 0.5 to 1 cm pieces which were transferred to callus induction medium.

MS medium [15], supplemented with 500 mg/l CH, was the basic culture medium for callus induction and regeneration of plants. Four mg/l 2,4-D were added to the MS medium for induction of callus and IAA at 0.5 mg/l and BA at 0.8 mg/l for regeneration of plants. Callus was initiated in the dark at 28°C. After calli formed they were subcultured on regeneration medium and transferred to an incubator with 16-hours of fluorescent light for plant regeneration. Calli induced from explants were transferred to regeneration medium at 2 to 3 weeks after callus formation. Plant regeneration was continued by subculturing on regeneration medium once a month. The frequency of callus induction and productivity of calli were used as estimates of induction efficiency. Frequency of plant regeneration was used as an

estimate of regeneration efficiency. These estimates were determined at each subculturing based on the following formulae:

$$(1).\ \text{frequency of callus formation} = \frac{\text{explants with callus}}{\text{no. of explants plated}}$$

$$(2).\ \text{callus growth index} = \frac{\text{total fresh weight of callus (g)}}{\text{no. of explants with callus}}$$

$$(3).\ \text{frequency of plant regeneration} = \frac{\text{regenerated plants}}{\text{no. of plated calli}}$$

Results

Callus initiation

The frequency of callus induction and callus growth indices are given in Table I. Callus production was the highest from immature panicles. When the explant was a mature embryo, callus formation was relatively high, but this callus grew poorly. Callus production was lowest from the mesocotyl, node, and mature panicle explants. Callus was not formed from endosperm of mature seeds.

Plant regeneration

Calli from immature panicle explants formed green spots, shoot or root formation, or sometimes a few plantlets when callus was transferred from explants to the regeneration medium. This was the only callus that formed organs or plantlets on the first subculture.

Plant regeneration was promoted by a second subculturing on fresh differentiation medium. The frequency of plant regeneration from calli derived from various explants was determined at the end of the second subculture (Table II). Regeneration frequency varied greatly with explant source. Calli from immature panicles gave up to 89% regeneration. The frequency was lowest for calli from roots and mature panicles. During the comparative studies of callus formation and plant regeneration 2,376 plants were regenerated from immature panicles of Labelle and Lemont (Table III), and a total of 5,773 plants have been regenerated in 2 years from the 12 rice cultivars used as explant sources.

Culture of immature panicles:

When immature panicles were demonstrated to be the best explant source for generation of callus and plants, the characteristics of callus induction and plant regeneration from immature panicles were studied further. Callus induction from immature panicles was first observed after 7 to 10 days incubation and continued to increase for about 45 days (Table IV). The growth rate was high for 4 weeks with callus produced at the rate of 72.6 mg/per day (fresh wt.). From 30 days to 45 days the growth rate slowed to 19.3 mg/per day. After 45 days of culture callus weight started to decrease. The ability of callus to generate plantlets from immature panicles of the cultivars Tetep and Tadukan was high until about 3 to 4 weeks in culture on callus induction medium (Fig. 1). Beyond this period the potential to regenerate plants was gradually reduced. After 10 weeks, few plants were produced.

External changes in callus growing on the regeneration medium were related to the ability of callus to produce plants. Calli could be separated into five types based on callus color and firmness (Table V). Most calli induced from immature panicles were firm, nodular, and yellow when transferred to regeneration medium. This type of callus had a high regeneration capacity and readily produced green spots. Calli producing green spots, or areas of high growth activity, actively regenerated plants. Over time this callus changed to a type which appeared yellowish to white,less firm, and had a lower frequency of plant regeneration. Soft, watery, brown callus derived from the previous types of calli were present after prolonged culture. The ability to regenerate plants was almost lost with this callus. The callus eventually became black and would not produce new callus or plants.

The ability of the calli to produce plants after subculturing was noted and listed in Table VI. Maximum plant regeneration occurred in the third subculture. As the callus quality declined in subsequent subcultures, the ability of callus to regenerate plants also declined. Few plants were regenerated in the fifth and sixth subcultures.

Several types of plants with chlorophyll deficiencies were regenerated in culture. These included plants with a general chlorophyll deficiency throughout the plant, albino plants, and variegated plants. The frequency of occurrence of these abnormal plants was 2.1% for plants with a general chlorophyll deficiency, 5.3% for albino plants, and 0.3% for variegated plants. Plants with a general chlorophyll deficiency survived and grew for a short time when transferred to pots, but then withered and died.

Discussion

Recently, plant regeneration from tissue culture has been greatly improved in gramineous crops by using immature meristematic tissues as the culture materials [16-19]. In a comparison of callus induction and plant regeneration from different explants in barley, Dale and Deambrogio [20] found that roots were easily regenerated from most explants. However, whole plants were regenerated only from callus from immature embryos. Our experimental results provided additional evidence that the immature panicle is the most effective explant source for rice somaculture. When immature panicles were used as the explant source, 55-90% more plants were regenerated compared to other explant sources. Large numbers of plants could be quickly regenerated from callus from immature panicles using a simple procedure involving slight modification of the MS medium and several callus subcultures.

Calli derived from root, mesocotyl, and mature embryo explants mainly produced roots but not whole plants. Calli derived from immature panicles actively produced shoots followed by rapid root formation. The frequency of regeneration of green plants from callus derived from immature panicles showed a significant decrease over time in this study and in other studies [12]. Part of the decrease was due to the fact that totipotent callus cells were utilized to regenerate plants and certain callus cells became brown or black and lost the capacity to regenerate plants. In carrot culture, Halperin introduced the concept of embryogenic competence in 1967 [21]. He referred to the capacity to regenerate plants as the inherent totipotency of the cultured cells. Street [22] advanced the concept that morphogenic

competence was determined by three factors: (1) the time of culture initiation, (2) the culture conditions leading to morphogenic expression of the competence, and (3) the loss of the ability to achieve organogenesis or embryogenesis in non-competent cells. In relation to this concept, the initial cultures in our system appeared to be a mixed population of competent and non-competent cells, that is, cells with or without the ability to generate embryos of plants. After prolonged subculturing, the non-competent cells gradually became dominant, eventually replacing competent cells in the population. Thus, plant regeneration could no longer occur. The cause of the reduction in number of competent cells and the increase in number of non-competent cells remains unknown. We are presently studying the histocytology of callus formation and plant regeneration from immature panicles for understanding the processes of dedifferentiation and redifferentiation in our culture system.

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Table I. Effect of explant source on induction of callus by the rice cultivars Labelle and Lemont.

Cultivar	Explant source	No. of explants cultured	Explants with callus	Frequency of callus formation (%)	Fresh weight of callus (g)	Callus growth index
Labelle	Immature panicle	62	53	85.5	47.2	0.89
	Root	62	58	93.5	32.4	0.56
	Mature Embryo	45	41	91.1	8.8	0.22
	Mesocotyl	35	23	65.7	4.7	0.20
	Node	61	37	60.7	4.4	0.12
	Mature panicle	71	18	25.4	4.5	0.25
	Endosperm	45	0	0	0	0
Lemont	Immature panicle	75	60	80.0	55.8	0.93
	Root	42	40	95.2	31.1	0.78
	Mature Embryo	57	52	91.2	13.6	0.26
	Mesocotyl	57	36	63.2	6.0	0.17
	Node	64	35	54.7	6.7	0.19
	Mature panicle	86	15	17.4	3.5	0.23
	Endosperm	52	0	0	0	0

Table II. Effect of explant source on plant regeneration by the rice cultivars Labelle and Lemont.

Cultivar	Explant source	No. of calli plated on regeneration medium	No. of plants regenerated	Frequency of plant regeneration (%)
Labelle	Immature panicle	74	66	89.2
	Node	90	35	38.9
	Mesocotyl	78	25	32.1
	Mature Embryo	67	12	17.9
	Root	79	6	7.6
Lemont	Immature panicle	70	51	72.9
	Node	92	30	32.6
	Mesocotyl	58	15	25.9
	Mature Embryo	68	6	8.8
	Root	73	0	0
	Mature panicle	72	6	8.3

Table III. Total number of Labelle and Lemont somaclones regenerated from different explant sources.

Explant source	Cultivar	
	Labelle	Lemont
Immature panicles	1,730	646
Mesocotyl	734	157
Nodes	289	171
Mature embryos	58	51
Mature panicles	-	91
Root tips	35	-

Table IV. Callus production over time from immature panicles of the rice cultivar Labelle.

Days on callus induction medium	7-15	15-30	30-45	45-60
Weight of callus (mg)	650	1,590	1,880	1,640
Increase in callus (mg)	650	940	290	-240
Growth rate (mg/day)	81	63	19	-16

Table V. Effect of callus quality on plant regeneration by the rice cultivar Lemont.

Callus quality	Firm yellow callus with green spots	Yellow firm granulated callus	Yellow callus	Yellow to white soft callus	Brown or dark callus
No. of calli transferred	52	34	59	29	20
No. of calli with shoots	46	14	16	2	0
Rate of shoot formation (%)	88.5	41.2	27.1	6.9	0
No. of regenerated plants	160	40	49	4	1
Frequency of regeneration (%)	307.7	117.6	83.1	13.8	5

Table VI. Effect of number of callus subcultures on plant regeneration with the rice cultivar Labelle.

No. of subcultures	No. of calli transferred	No. of plants regenerated	Frequency (%)
2	119	98	82.4
3	139	152	109.4
4	87	59	67.8
5	37	12	32.4
6	15	4	26.7

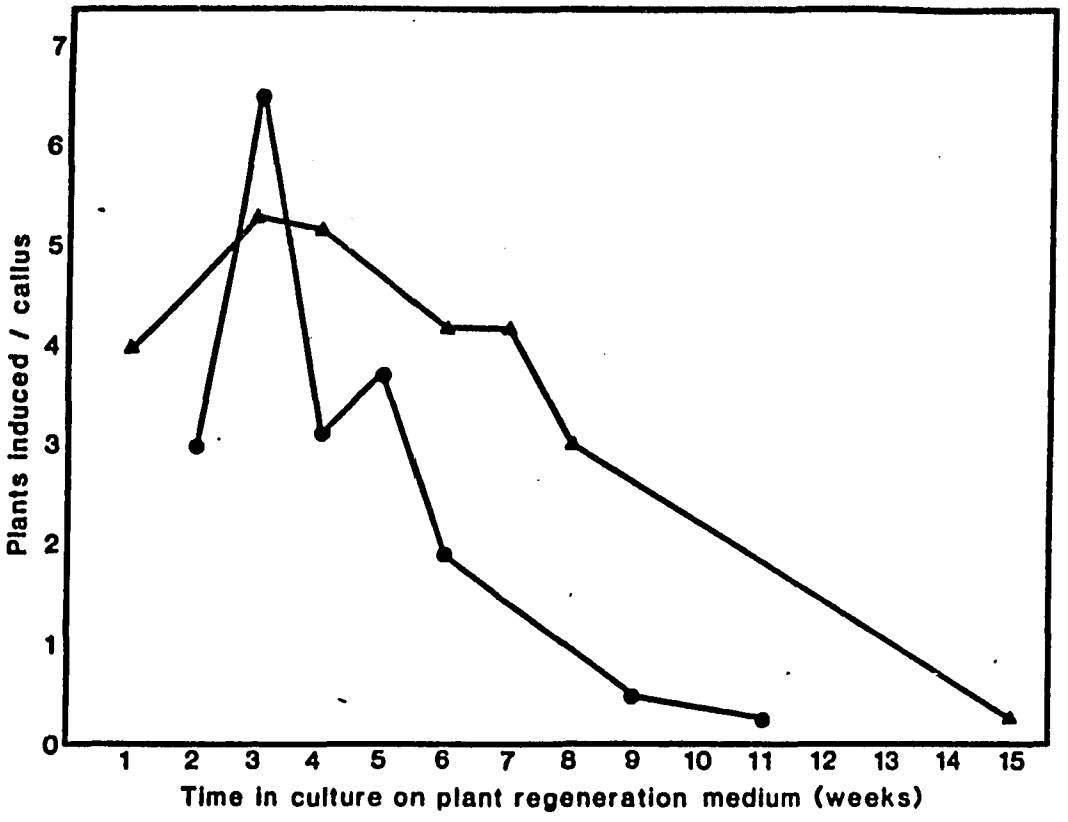

Fig. 1. Plant regeneration over time from immature panicle explants from the cultivars Tetep ▲ and Tadukan ● .

CHAPTER II

EFFECT OF CULTIVAR, NUTRITION, AND CULTURE CONDITIONS ON RICE SOMACULTURE

ABSTRACT

Cultivars, media, growth regulators, sucrose concentration, photoperiod, temperature, and inoculum size were evaluated for their effects on callus induction and plant regeneration in rice somaculture. Plants were readily regenerated from the cultivars Tetep, Tadukan, Labelle, and Lemont. The auxin 2,4-D was necessary to initiate callus induction. The combinations IAA (0.5 mg/l) and BA (0.8 mg/l) or NAA (2 mg/l) and Ki (1 mg/l) in regeneration media gave the best plant regeneration. The optimum concentration of sucrose for callus formation and plant regeneration was 3%. Experiments for testing the effects of light and temperature indicated that for callus induction explants should be cultured in the dark at 28°C. For plant regeneration, callus should be cultured at 28°C under fluorescent light. The initial size of callus pieces transferred from the explant affected callus growth and plant regeneration. Growth rate of calli and plant regeneration were inversely related to size of the callus piece transferred.

Abbreviations: BA, N^6-benzyladenine; IAA, indole-3-acetic acid; Ki, kinetin; LS, Linsmaier and Skoog; MS, Murashige and Skoog; NAA, a-naphthaleneacetic acid; R_2, Chalef's R-2; 2,4-D, 2,4-dichlorophenoxyacetic acid.

Introduction

It is well known that the processes of dedifferentiation and redifferentiation in plant tissue culture depend on the interaction of internal growth factors and external environmental factors. Effects of genotypes, nutritional requirements, and culture conditions have been widely studied and well documented in both dicots and monocots [1-3]. It has been observed in rice that variation in callus formation and plant regeneration depends on species, subspecies, and the particular cultivars used [4-6]. Investigations on manipulation of nutritional requirements of the medium have shown that the growth rate and plant regeneration of cultured cells could be regulated by changing the medium [7-9]. Conger [10] and Oono [11] have reviewed methods for *in vitro* culture of rice. Recently, Raghavaram and Nabors [12] found that initial inoculum size could affect plant regeneration from seed-derived embryogenic callus of rice.

Our intention in this study was to determine the optimum nutritional and physical requirements for somaculture of rice using immature panicles as the explant source.

Materials and methods

Plant material

Immature panicles of the cultivars CICA 6, Labelle, Lebonnet, Lemont, Saturn, Tadukan, and Tetep were used as experimental materials for initiation of callus and plant regeneration. Rice tillers with unemerged immature panicles, 1-6 cm in length and yellowish or white in

color, were collected from plants grown in the greenhouse. The tillers were thoroughly washed in running tap water. After removing the outer leaf sheaths, the boots from each tiller were sterilized in 1.6% sodium hypochloride solution for 30 minutes and rinsed three times with sterile water. The immature panicles were dissected, cut into 0.5 to 1.0 cm pieces using sterile technique, and transferred to modified MS medium [13].

Culture medium and conditions

The general procedure for callus induction and plant regeneration from immature panicles (IP) was as follows:

(a) Hormones. The auxin 2,4-D was added to the basic medium at 0, 0.5, 1, 2, 3, 4, and 5 mg/l. Various combinations and concentrations of NAA and Ki were added to the MS medium. IP explants were transferred to the media supplemented with the different hormones at various concentrations (Tables III and IV) and the success of callus induction on each medium was determined after 30 days incubation at 28°C in the dark.

Callus from IP explants were transferred to MS based media containing different combinations and concentrations of IAA, NAA, Ki, and BA (Table V). The frequency of plant regeneration was determined after 30 days.

(b) Media. Five basic media were compared for induction of callus from IP explants. These included MS, LS [14], N_6 [15], R_2 (pers. comm., see Rush and Shao) [16], and B_5 [17].

(c) Sucrose. Sucrose concentrations of 0, 1, 3, 5, 7, 10, and 15 per cent were prepared by addition of 0, 10, 30, 50, 70, 100, and 150

grams of sucrose to 1 liter MS medium with 2,4-D (4 mg/l) for callus induction and MS medium with BA (0.8 mg/l) and IAA (0.5 mg/l) for plant regeneration.

(d) Temperature. IP explants were incubated in the dark on MS medium supplemented with 2,4-D (4 mg/l) at 20, 24, 28, and 32°C, respectively, for 30 days, and callus production was recorded.

(e) Light. An incubator was programed for four intervals of dark/light periods. The dark/light (fluorescent) periods tested were 24/0, 16/8, 8/16, and 0/24. IP explants or calli from IP explants were incubated at these dark/light periods for evaluation of callus formation and plant regeneration. The production of callus and frequency of plant regeneration were determined over a 30 day period.

(f) Inoculum size. Two thousand calli each of 20, 50, 100, and 200 mg size were plated on MS medium containing 2,4-D (2 mg/l) for callus growth and MS supplemented with BA (0.8 mg/l) and IAA (0.5 mg/l) for plant regeneration. The relation between callus initial weight and growth rate or plant regeneration was determined at the end of the subculture period.

Results and Discussion

Cultivar

Plant regeneration began with the initiation of shoot formation. The potential of plant regeneration varied among the cultivars. There was variation in regeneration frequency and the subculture in which plants were regenerated (Table I). When calli were transferred from IP explants to regeneration medium, callus from Tetep and Tadukan underwent

active differentiation. Most of the transferred calli developed shoots. Plantlets covered the surface of the medium after one month of incubation. Transfer of callus from which plants were regenerating to fresh medium led to further growth of callus and further regeneration of plants. When callus from IP explants from Labelle and Lemont were transferred to regeneration medium, only a few plantlets were regenerated by the end of the first subculture. The number of plants regenerated was high during the second subculture. Most of the calli from Lebonnet, Saturn, and CICA 6 IP explants produced roots on the differentiation medium in the first subculture. Transfer of these rooted calli to fresh medium led to blackening and death of the callus. Only a few calli produced shoots and developed into plants. Similar differences among cultivars for plant regeneration were previously reported for Oryza glaberrima Steud by Fatokun and Yamada [5]. Calli derived from 16 of 20 cultivars gave regenerated plants in their study. Apparently, different genotypes have different physiological requirements for plant regeneration. Manipulation of culture media should allow different genotypes to regenerate plants more efficiently.

Media

Callus could be produced by IP explants on all of the media tested. There was little difference in the frequency of callus induction and growth rate among the media (Table II). These results suggest that IP explants had general adaptability to culture media for callus induction.

Hormones

Concentrations of 2,4-D from 1 to 5 mg/l gave 100% of PI explants producing callus (Table III). In order to induce callus formation, the medium had to contain 2,4-D. Callus induction was less effective at 0.5 mg/l 2,4-D. Increasing the 2,4-D level increased the amount of callus produced in the 2 to 4 mg/l 2,4-D range (Table III).

The combinations and concentrations of NAA and Ki added to MS medium are shown in Table IV. The NAA was maintained at 2.0 mg/l with Ki concentrations of 0.1, 0.5, and 1.0 mg/l. Callus induction was reduced compared to media with 2,4-D. As the concentration of Ki increased callus formation was inhibited. With Ki in the medium, many immature embryos developed from immature panicles and a few plants were regenerated. More plants were regenerated from callus from Labelle and Lemont IP explants on medium containing Ki at 0.5 mg/l. One plant was regenerated directly from a Lemont IP explant without formation of callus. Similar results were reported for callus induction from cotyledonary nodes of the rice cultivar Taichung No. 65. by Wu and Li [18] and from seeds by Yamada, et al. [19].

Various combinations and concentrations of IAA, NAA, Ki, and BA were added to MS medium for testing their effects on plant regeneration (Table V). The frequency of plant regeneration was low on the medium without growth regulators. Plant regeneration from calli was most effectively promoted by media containing either IAA (0.5 mg/l) and BA (0.8 mg/l) or NAA (2 mg/l) and Ki (1 mg/l).

Sucrose

Callus formation was completely inhibited in the medium without sucrose (Table VI). Induction of callus was also significantly reduced

at high levels of sucrose. Generally, callus could be induced from IP explants in the range of 1 to 10% sucrose. The optimum level for callus induction was at about 3% sucrose.

Response of plant regeneration to sucrose concentration was much more sensitive than callus induction (Table VII). No plants could be regenerated from calli if the culture medium was not supplied with sucrose. On the other hand, plant regeneration was inhibited completely by 15% sucrose. Maximum plant regeneration occurred over the range of 3 to 5% sucrose.

Photoperiod

Rice IP explants showed sensitivity to photoperiod in callus formation (Table VIII). The inhibitory effects of light increased as the photoperiod increased. Callus growth was enhanced by dark with three times as much callus produced in the dark compared to callus production in continuous light.

Although light was not necessary for callus induction and growth, shoot formation and plant regeneration were stimulated by light (Table IX). The number of plants regenerated was highest with a 16 hour light / 8 hour dark regime.

Temperature

Callus was induced from IP explants at all of the temperatures tested (Table X). However, great differences were observed in callus production. The optimum temperature for callus growth was 28^{o}C.

Weight of callus piece transferred

Callus growth and plant regeneration in rice from calli generated from IP explants was inversely related to initial weight (Table XI, XII). Experiments on the effects of initial size of the callus piece transferred on subsequent growth of the callus were studied in tissue culture of Jerusalem artichoke and carrot by Caplin [20] and in Corchorus and Nigella by Bhattacharya, et. al., [21]. Raghavaram and Nabors [12] reported on the effects of initial callus size on plant regeneration in rice. It was concluded from these experiments that a relationship exists between the initial size of a tissue mass and subsequent callus growth and frequency of plant regeneration. It is known that cells at the surface periphery of a callus mass have higher potential for cell division, enlargement, and differentiation. Cells in the interior of the callus mass appear to be less active in growth and development. There are proportionally more peripherial cells in a small callus mass than in a larger mass.

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Table I. Cultivar effects on plant regeneration from callus produced on immature panicle explants in rice.

Cultivar	No. of calli transferred from IP explants	Total No. of plants regenerated	Frequency of regeneration (%)
Cica-6	87	11	12.6
Labelle	54	94	174.1
Lebonnet	90	63	70.0
Lemont	52	77	148.1
Saturn	91	35	38.5
Tetep	30	87	290.0
Tadukan	42	95	226.2

Table II. Effects of media on callus formation by immature panicle explants from the rice cultivar Lemont.

Media	No. of plated immature panicles	No. of explants forming callus	Percentage of explants forming callus (%)	Fresh weight of callus (g)	Callus weight per explant piece (g)
LS	10	9	90.0	23.8	2.7
MS	11	11	100.0	28.5	2.6
B_5	8	7	87.5	17.8	2.5
N_6	7	6	85.7	14.7	2.5
R_2	12	11	91.7	29.1	2.7

Table III. Effect of 2,4-D concentration on callus formation by immature panicle explant tissue from the rice cultivars Labelle and Lemont.

Cultivar	2,4-D (mg/l)	No. of immature panicles	No. of explants forming callus	Frequency of callus formation (%)	Fresh weight of callus formed (g)
Lemont	0	14	0	0	0
	0.5	12	11	92	7.0
	1	10	10	100	15.3
	2	11	11	100	18.4
	3	8	8	100	14.1
	4	7	7	100	14.2
	5	6	6	100	9.3
Labelle	0	11	0	0	0
	0.5	10	9	90	4.7
	1	7	7	100	6.9
	2	10	10	100	13.0
	3	10	10	100	14.1
	4	9	9	100	15.0
	5	8	8	100	10.5

Table IV. *In vitro* response of immature panicle explants of the rice cultivars Labelle and Lemont to NAA and Kinetin in MS medium on callus induction and plantlet regeneration.

Cultivar	Growth regulators (mg/l) NAA	Ki	No. of panicles plated	No. of panicles producing callus	Frequency of callus induction (%)	No. of calli regenerating plants	Frequency of plant regeneration (%)	No. of regenerated plants
Labelle	2.0	0.1	10	3	40.0	1	10.0	1
	2.0	0.5	11	4	72.7	4	36.4	4
	2.0	1.0	9	1	33.3	2	22.2	2
Lemont	2.0	0.1	10	3	40.0	1	10.0	1
	2.0	0.5	13	2	30.8	2	23.1	4
	2.0	1.0	10	1	10.0	0	0	0

Table V. Effect of growth regulators on plant regeneration from calli induced from immature panicle explants from the rice cultivar Lemont.

Growth regulators (mg/l)	No. of calli plated	No. of plants regenerated	Frequency of regeneration (%)
None	44	8	18.2
IAA 0.5 + BA 0.8	33	20	60.6
IAA 0.5 + BA 1.6	42	19	45.2
IAA 0.5 + BA 3.2	44	22	50.0
IAA 0.5 + Kinetin 0.8	47	8	17.0
IAA 0.5 + Kinetin 1.6	46	6	13.0
IAA 0.5 + Kinetin 3.2	50	16	30.0
NAA 2.0 + Kinetin 0.5	54	15	27.8
NAA 2.0 + Kinetin 1.0	51	38	74.5
NAA 2.0 + Kinetin 2.0	57	13	22.8

Table VI. Effect of sucrose concentration on callus formation from immature panicle explants from the rice cultivar Lemont.

	Sucrose concentration % (w/v)						
	0	1	3	5	7	10	15
No. of explants inoculated	7	6	9	6	8	6	5
No. of explants with callus	0	5	9	6	8	6	2
Frequency of callus formation (%)	0	83.3	100	100	100	100	40
Total fresh weight of callus (g)	0	5.5	20.7	9.9	11.7	6.6	1.3

Table VII. Effect of sucrose concentration on plant regeneration from calli produced on immature panicle explants from the rice cultivar Lemont.

	Sucrose concentration % (w/v)						
	0	1	3	5	7	10	15
No. of calli plated	24	22	25	25	27	26	20
No. of regenerated plants	0	1	17	18	6	1	0
Frequency of regeneration (%)	0	4.5	68	72	22.2	3.8	0

Table VIII. Effect of photoperiod on callus formation from immature panicle explants from the rice cultivar Labelle.

Photo-period (hr)	No. of IP explants	No. of explants with callus	Frequency of callus formation (%)	Fresh weight of callus (g)
0	22	14	63.6	12.9
8	17	9	52.9	7.5
16	23	12	52.2	7.0
24	21	7	33.5	3.9

Table IX. Effect of Light on plant regeneration from immature panicle explant calli produced by the rice cultivar Labelle.

Lighted period (hr)	No. of calli plated	No. of calli with plantlets	Percentage of calli with plantlets (%)	No. of regenerated plants	Plant regeneration (%)
8	201	102	50.7	133	66.2
16	119	76	63.9	98	82.4
24	154	85	55.2	99	64.3

Table X. Effect of temperature on induction of callus from immature panicle explants from the rice cultivar Lemont.

Temperature (^oC)	No. of immature panicles plated	No. of explants with callus	Percentage of explants forming callus	Fresh weight of callus (g)
20	16	14	87.5	7.3
24	14	12	85.7	7.2
28	11	9	81.8	13.0
32	12	10	83.3	10.6

Table XI. Relationship between the size of the callus piece transferred and growth rate in the rice cultivar Lemont.

	Initial callus size[a]			
	0.02	0.05	0.1	0.2
Mean of fresh weight (after 30 days)	33.24	31.10	13.70	10.90
Growth rate (g/day)	1.11	1.07	0.46	0.36

[a] Two thousand callus pieces were transferred for each callus size.

Table XII. Relationship between the weight of the callus piece transferred to plant induction medium and plant regeneration with the rice cultivar Lemont.

	Callus weight at transfer (g)[a]			
	0.02	0.05	0.1	0.2
No. of regenerated plants	53	25	12	5
Frequency of regeneration (%)	27.0	12.5	6.0	2.5

[a] Two thousand callus pieces were transferred for each callus weight.

CHAPTER III

MORPHOLOGY AND HISTOLOGY OF CALLUS FORMATION AND PLANT REGENERATION FROM IMMATURE PANICLES IN RICE (*Oryza sativa* L.)

ABSTRACT

The origin of callus tissue and the pattern of *in vitro* development and regeneration of plants initiated from immature panicles of rice (*Oryza sativa* L.) were studied histologically. Callus originated from spikelets primarily through growth of cells from floral primordia. Callus tissues were also endogenously induced from the peduncle (neck), rachis, and branches in panicles. Compact callus, when transferred to MS medium supplemented with 0.5 mg/l (IAA) and 0.8 mg/l (BAP), regenerated plants through both embryogenesis and organogenesis. Shoot primordia and embryo-like structures spontaneously arose *de novo* from calli. A mixture of various embryogenic and organogenic structures were present in the same callus. Differentiation of root primordia was characterized by acquisition of dense cytoplasmic substances by parenchyma cells which proceeded active division.

Abbreviations: 2,4-Dichlorophenoxyacetic acid = 2,4-D. Benzylaminopurine = BAP. Indole-3-acetic acid = IAA. Murishige and Skoog's medium = MS.

1. Introduction

Recent advances in plant tissue and cell culture in gramineous plants have led to much speculation about ways in which cereal crop plants can be regenerated through tissue culture. In rice callus culture, it has been reported that plants could be regenerated from various explant sources either through organogenesis or by embryogenesis (Tamura 1968, Nakano and Madea 1979, Genovesi and Magill 1982, Abe and Futsufara 1985). However, histological observations were made in only a few cases. It was shown that plant regeneration from mature embryo-derived callus was through organogenesis (Tamura 1968). Plant regeneration from anther or root derived callus was through embryogenesis (Genovesi and Magill 1982, Abe and Futsufara 1985). Detailed histological studies of callus formation and plant regeneration from immature panicles have not been carried out. Such information can be of critical value in understanding the process of plant regeneration, as well as, useful in somatic genetics and crop improvement.

We have established an efficient culture system and thousands of plants have been regenerated through the culture of immature rice panicles. In this study the morphological and histological events in callus formation and plant regeneration from immature panicles were described.

2. Materials and Methods

2.1 Initiation of Callus Formation

Immature, unemerged panicles (1-6 cm in length) of rice were obtained from field grown plants. After removing the outer leaf

sheaths, the boot from each tiller was sterilized in 1.6% sodium hypochloride solution for 30 minutes and then washed three times with sterile deionized water. The immature panicles were dissected out using sterile technique, cut into 5 mm to 10 mm segments, and placed onto modified MS medium supplemented with 500 mg/l casein hydrolysate, 30 g/l sucrose, 10 g/l agar and 4 mg/l 2,4-D. The pH of the medium was adjusted to 5.8 before autoclaving. The cultures were incubated in the dark at 28°C in a growth chamber.

2.2 Plant regeneration

The yellow compact tissue induced from pieces of immature panicle plated onto induction medium were transferred to the differentiation medium. The differentiation medium was the same as the induction medium except for the addition of 0.4 mg/l BAP and 0.5 mg/l IAA instead of 2,4-D. The cultures were maintained at 28°C, with 16 hr light and 8 hr dark, and subcultured every 4 weeks. Plants were actively regenerated from the second subculture through subsequent subculturing.

2.3 Histology

Explants forming callus on the induction medium and calli producing shoots in the second subculture on differentiation medium were prepared for light or scanning electron microscopy.

The explants with callus or callus with tiny green protrusions were fixed in FAA (90 ml of 70% ethanol, 5 ml of formaldehyde, and 5 ml of glacial acetic acid) and dehydrated in gradual steps in a tertiary-butyl alcohol series. Specimens were then embedded and cast in plastic polymers in Paraplast. Following embedding, specimens were

sectioned at 7 um with a steel blade on an American Optical Rotary Microtome and mounted in sequence on glass slides. After mounting, the sections were deparaffinized, stained in a safranin-fast green staining series and made permanent with Permount.

Cultured tissues were also fixed by overnight incubation in 3% glutaraldehyde in 0.1 M sodium phosphate buffer (Ph 7.1). Following fixation, specimens were washed in the same buffer and dehydrated gradually in 30%, 50%, 70%, 95%, and 100% ethyl alcohol. Specimens were dried in a Polaron Critical Point Drying Apparatus, using liquied CO_2, and coated with gold/palladium (200A) using a sputter coating unit (Polaron). Specimens were scanned at 25 KV in a Hilton S-500 scanning electron microscope.

3. Results

3.1 Callus formation

Development of rice panicles is characterized by constant and sequential changes in the pattern of organ initiation. Primary branches born on the rachis of the panicle give rise to secondary branches which, in turn, produce spikelets. The major parts of the panicle present as explants at the time of excision and culture were pieces of panicle peduncle, rachis, branches, and spikelets.

Spikelet explants showed a central core of large, highly vacuolated parenchymatous cells surrounded by an outer mantle (Fig.1). Small vascular bundles were embedded in the parenchymatous cells (Fig.2).

Very little starch was present in these vacuolated parenchyma cells. Cells of the peripheral region appeared to be meristematic cells

with rich cytoplasm and showed active division after the explants were cultured on the medium containing 2,4-D (Fig.1). Callus formation occurred from these regions. Callus tissues first became visible on explants 7 days after culture on MS medium containing 2,4-D. The majority of calli originated from spikelets (Fig.3). Callus was formed, however, on panicle peduncles (necks), rachis, and primary and secondary branches (Fig.4).

A longitudinal section of rachis is illustrated (Fig.5). The vascular cylinder appeared as a column surrounded by ground tissue composed of parenchymatous cells in longitudinal files. Epidermis occupied the outer most layer. Longitudinal views showed that conspicuous nuclei were present in the parenchymatous cells surrounding the vascular bundle (Fig.6). The cytoplasm of these cells became dense and the cells were actively dividing. The resulting callus tissue penetrated the ground tissues, ruptured the epidermis, and emerged on the surface of the explant (Fig.7). Callus derived from immature panicle explants was yellow and compact (Fig.8). The callus consisted of small, round cells, with the potential for plant regeneration (Fig.9). Callus showing these morphological characters was transferred to differentiation medium for plant regeneration.

3.2 Plant regeneration

The callus, when transferred to the differentiation medium, proceeded to redifferentiate and produce shoots and roots. The calli appeared green and initiated shoot formation in the first subculture. Continued subculture of the green callus tissue led to prolific regeneration of plants. The calli initiating plantlets were used for

histological observations to determine differentiation pathways in the process of plant regeneration.

(a) Embryogenesis

Calli differentiating by embryogenesis showed the formation of globular embryoids (Fig.10). Later, somatic embryoids with a well defined scutellum, coleoptile, and coleorhiza were observed (Fig.11-12). The emergence of heart shaped structures on the surface of the callus was the first sign of the early developmental stages of embryogenesis (Fig.13). Continued division and organization in some of these structures gave rise to preembryoids attached to the callus by a suspensor (Fig.14). The basal end of the preembryoids showed vaculated cells embedded in the callus and a rounded tip end containing small and densely cytoplasmic cells without intercellular spaces. Advanced differentiation led to the formation of a lateral notch in the terminal end (Fig.15). Further organization resulted in the formation of distinct bipolar embryoids with a scutellum, a coleoptile, and a coleorhiza (Fig.16).

(b) Organogenesis

An early morphological characteristic of calli initiating organogenesis was the emergence of tiny protuberances on the surface (Fig.17). Later organization of the shoot apex gave rise to leafy structures and leaf primordia (Fig. 18). Eventually, new plantlets became visible from the tiny green protrusions (Fig. 19). Sections of callus grown on the differentiation medium showed that cells in different locations were morphologically distinct (Fig.20). The outer

most cells were arranged in a surface layer. Subsurface and innermost regions were composed of parenchyma cells in more or less continuous layers or in strands. These cells appeared highly vacuolated. Between the subsurface and innermost regions was a meristematic region. It was considered the subperipheral region. These cells appeared small and rich in cytoplasm and contained a permanent nucleus and nucleolus (Fig.21). This region was similar to procambium in structure and cells in this region showed meristematic activity. Meristematic cells became evident in various, apparently random, locations on the surface of callus tissues. This was probably due to the preferential activity of cells in the subperipheral region (Fig.20). Mitotic activity of cells in these areas was increased when compared to surrounding areas. Development of these areas began with the appearance of a protuberance on the tissue surface (Fig.22). A strand of procambium developed in the center of the protuberance (Fig.23). Meristematic cells in the peripheral region proceeded to develop a shoot apex (Fig.24). The organization of *in vitro* induced shoot apexes appeared very similar to the arrangement of development of shoot apexes *in vivo* (Fig.25). The shoot apex showed a one layer tunica with enclosed corpus and peripheral emergence of leaf primordia.

It was observed that spontaneous development of embryogenic or organogenic structures occurred within the same callus with concomitant development of plantlets (Fig. 26 and 27).

(c) Rhizogenesis

When a root was first initiated within callus tissue growing on differentiation medium, several parenchyma cells within the callus

acquired dense cytoplasm followed by active division (Fig.28). The accumulating cells gave rise to a protrusion, the root primordia (Fig.29). During growth of the young primordia through the callus, the apical meristem and root cap were initiated and the vascular system and cortex formed behind the apical meristem (Fig.30). Adventitious roots regenerated in vitro also showed characteristic arrangements of cells in the root tip, more or less similar to that of roots formed in vivo. The apical meristem region was composed of a root cap, a quiescent center, an epidermis, a cortex, and a vascular cylinder (Fig.31).

(d) Xylogenesis

Vascular bundles were often observed embedded in parenchyma cells throughout the callus proceeding redifferentiation on the differentiation medium (Fig.32). Sections of differentiating callus revealed xylem differentiating in the form of tracheids which were annular, spiral, and had pitted thickenings on their walls (Fig.33-34) Development of tracheary elements was observed between areas in which shoot or root meristems were located (Fig.35).

(e) Senescence

Callus growing on differentiation medium tended to turn brown in prolonged culture. The potential for plant regeneration was lost. Histological observation of these brown tissues showed that parenchyma cells with cytoplasmic substances became vacuolated with loss of starch grains after prolonged culture (Fig. 36). These cells became deformed, were crushed, and pushed aside (Fig.37).

4. Discussion

The inflorescence of gramineous plants has been found to be a good explant source for generation of callus. The floral primordium in Pennisetum americanum (Botti and Vasil 1983) or rachis Pennisetum purpureum (Wang and Vasil 1982) or both in Sorghum bicolor (Brettell et al. 1980) were shown to be excellent explants for callus formation. Culture of immature panicles of rice have shown that callus was generated from spikelet, peduncle, rachis, and branches in our studies. Histological evaluation of callus formation showed that meristematic activity at the periphery of floral primordium contributed to callus formation from spikelets. Callus was endogenously induced from the rachis and panicle branches.

Two distinct pathways,organogenesis and embryogenesis, have been demonstrated for plant regeneration. Nakano and Maeda (1979) described organogenesis in seed-derived callus in rice. In their view, the initiation of organized development in the callus was characterized by the formation of a radial file in the peripheral region. This was followed by development of a protuberance on the surface layer and formation of leafy structures.

The recent emphasis on cytohistological differentiation in gramineous species has been on embryogenesis, whether through the culture of immature embryos (Vasil and Vasil 1982), or the inflorescence (Botti and Vasil 1983, Boyes and Vasil 1984). In general, the sequence of embryogenesis was described as the separation of embryogenic cells, internal divisions, and formation of bipolar embryoids (Botti and Vasil 1983).

Whether in vitro plant regeneration is through embryogenesis or

organogenesis depends on the morphological characteristics, physiological, and genetic potential of cells, as well as, their interaction with environmental conditions. Cells in different locations or positions in a plant tissue show differences in physiological trends and, therefore, different response to specific in vitro culture conditions. In a review of developmental pathways for plant regeneration, Thorpe (1980) pointed out that it was the degree of dedifferentiation in meristematic cells that determined whether organs or somatic embryos were formed. This view holds that if dedifferentiation is complete, embryos will be formed; whereas, partial dedifferentiation will lead to the formation of organ primordia. Diversity of dedifferentiation and subsequent redifferentiation could allow the organization of various structures by embryogenesis or organogenesis in the same callus. This was in fact observed in our studies of rice.

Plant regeneration through both organogenesis and embryogenesis within the same culture have been demonstrated in Leucosceptrum canumsm (Pal et al. 1985), Cyclamen persicum (Wicart et al. 1984), Zea mays (Miguel 1984), Sorghum bicolor (Dunstan et al. 1978, 1979, Wernicke et al. 1982), and Dimorphoheca ecklonis (Anand and Mehra 1982). Based on their experiments, Wicart et al. (1984) suggested that the organogenic pattern in C. persicum could well be converted to a pathway for somatic embryogenesis in culture.

It has been reported that conversion or alteration of developmental pathways does occur in some cases. When studying plant regeneration from the inbred line B73 in Zea mays, Lower et al. (1985) isolated an embryogenic callus line from organogenic tissue cultured

for 2 years. The effects of genetic factors on the developmental pathway have recently been stressed. Abe and Futsufara (1984) analyzed sixty rice cultivars for their response to *in vitro* differentiation and plant regeneration. They found that different cultivars showed large differences, not only in potential for plant regeneration, but also in the pattern of organization for plant regeneration. Several cultures from *indica* cultivars were found to initiate plantlets through somatic embryogenesis.

The present study provides evidence that both pathways were involved in the process of redifferentiation during culture. The presence of globular structures, formation of a lateral notch, and the development of bipolar embryoids were considered as evidence for somatic embryogenesis in callus tissue derived from immature panicles. Organogenesis was characterized by unipolar redifferentiation with organ formation.

The significance of whether the plants were produced by embryogenesis or organogenesis in this somaculture system lies with the potential for somaclonal variation. In embryogenesis the plant is assumed to arise from a single cell. In organogenesis the plant may arise from several cells. The effects of these pathways on the amount of somaclonal variation in regenerated plants is not known at this time. If greater variation is possible through one pathway or the other, it should be possible to manipulate the culture conditions so as to favor one pathway over the other and to maximize or minimize variation. Under conditions where plants are to be reproduced by cloning, variation must be kept to a minimum. When somaculture is used to generate variation, callus cultures should be manipulated to

increase variation.

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Fig. 1. Cross section of spikelet of Oryza sativa L. at time of culture (x 63).

Fig. 2. Transection showing tracheids of xylem embedded in parenchyma cells (arrow) (x 160).

Fig. 3. Callus formation from spiklet. (x 16).

Fig. 4. Longitudinal section of rachis of Oryza sativa L. at the time of culture (x 160).

Fig. 5. Origin of callus from rachis (x 16).

Fig. 6. Longitudinal section of the rachis showing parenchyma cells with nucleus (n) and tracheids (tr) in callus (c) formation (x 400).

Fig. 7. Longitudinal section showing callus (c) formation from rachis with tracheids (tr) (x 160).

Fig. 8. Scanning electron micrograph of compact callus (x 30).

Fig. 9. Scanning electron micrograph showing mass of round parenchyma cells in the compact callus (x 150).

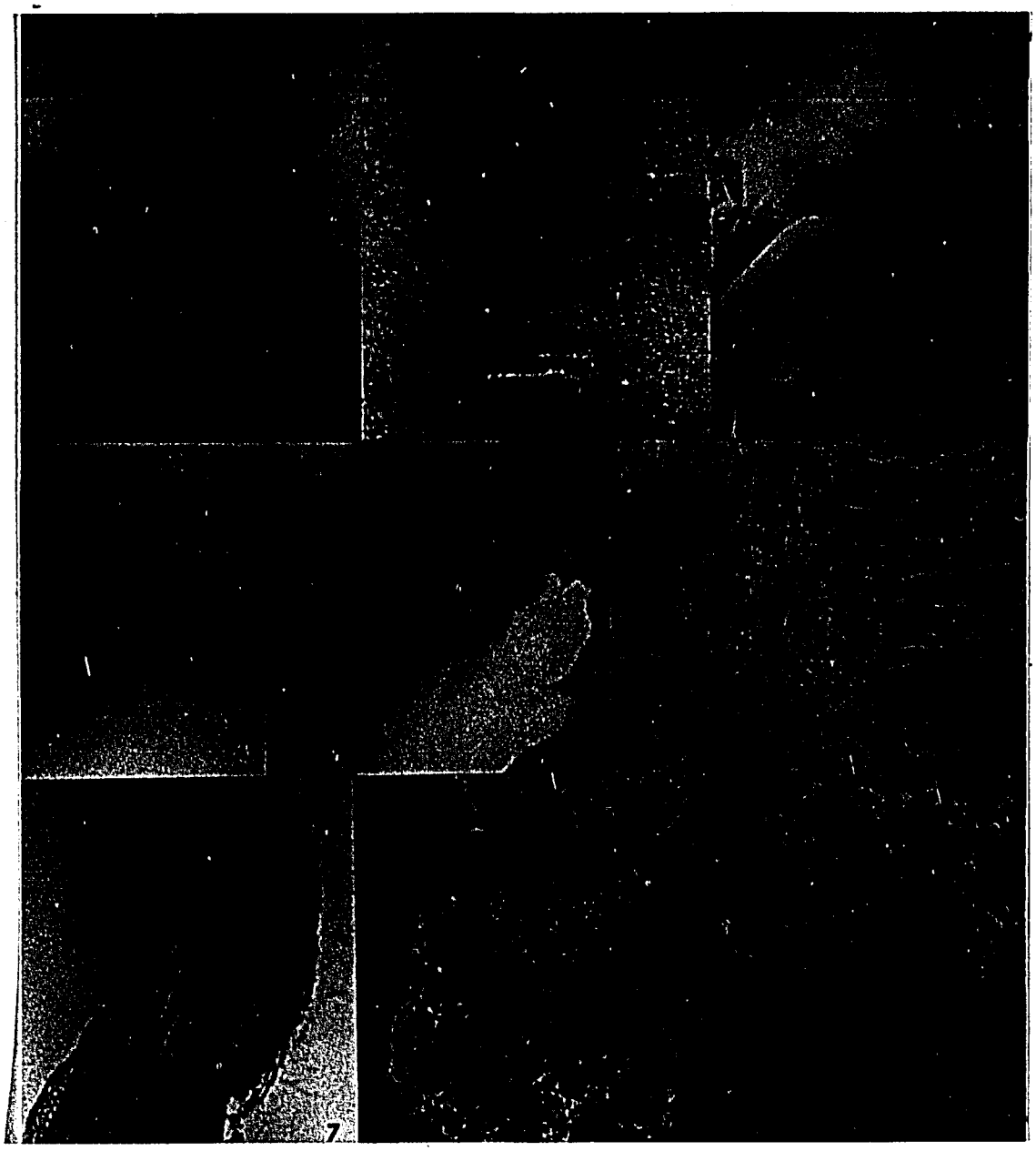
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Fig. 10. Scanning electron micrograph showing a region with many globular embryoids (g) and formation of a notch (arrow) (x 70).

Fig. 11. Scanning electron micrograph of development of embryoid with a scutellum (sc) and coleoptile (cl) (x 95).

Fig. 12. Scanning electron micrograph showing organized bipolar embryoid with a scutellum (sc), coleoptile (cl), and coleorhiza (cr) (x 90).

Fig. 13. Longitudinal section of globular embryoid (x 400).

Fig. 14. Longitudinal section of globular embryoid (g) with well defined epidermis (e) and suspensor (s) (x 160).

Fig. 15. Development of a notch (arrow) at the terminal end of an embryoid (x 160).

Fig. 16. Later stage of embryoid development with formation of a scutellum (sc), coleoptile (cl), and coleorhiza (cr) (x 400).

Fig. 17. Scanning electron micrograph showing emergence of protuberance on callus surface (x 150).

Fig. 18. Development of leaf primordium (Lp) and leaf (L) with vascular bundle (v) from shoot apex (x 200).

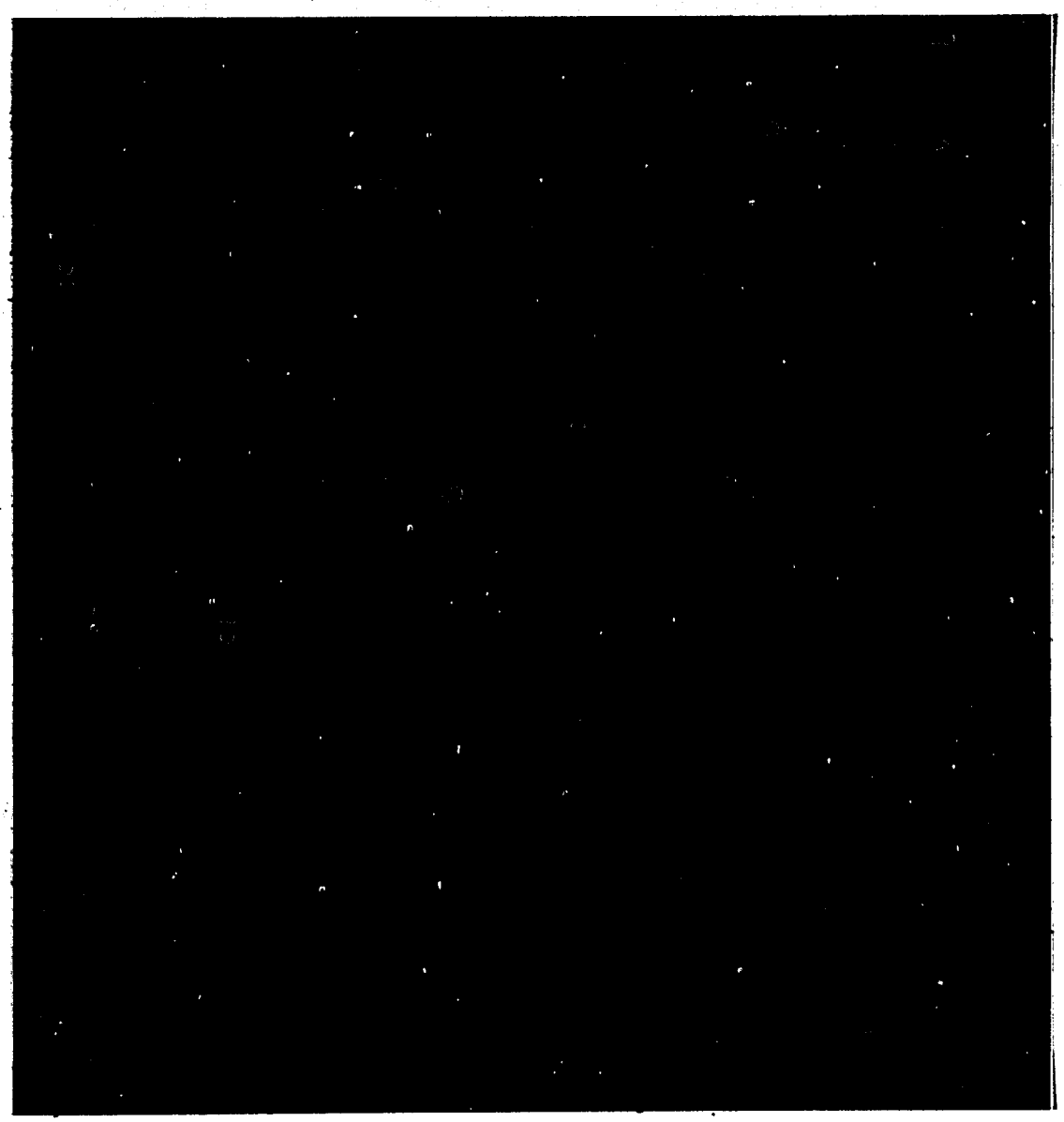


Fig. 19. Scanning electron micrography showing formation of plantlet from green protuberances.(x 20).

Fig. 20. Cross section showing epidermis (e), meristematic cells (mc) in procambium like layers, and parenchyma cells (pc) in callus proceeding organogenesis (x 63).

Fig. 21. Detail of meristemtic cells (mc) in procambium like layers and parenchyma cells (pc) (x 160).

Fig. 22. Longitudinal section showing development of protuberance (x 160).

Fig. 23. Longitudinal section showing initiation of a strand similar to procambium in the center of the protuberance, (arrow) (x 160).

Fig. 24. Longitudinal section showing development of shoot apex (sm) and leaf primordia (Lp) (x 160).

Fig. 25. Organization of *in vitro* induced shoot apex (sm), corpus (c), leaf primordium (lp), and tunica (t) (x 400).

Fig. 26. Longitudinal section showing initiation of both embryogenesis and organogenesis within same callus, formation of preembryoid (pe) and protuberance (p) (x 160).

Fig. 27. Longitudinal section showing initiation of both embryogenesis and organogenesis within same callus, formation of preembryoid (pe) and leafy-like protuberance (p) with development of vascular bundle (v) (x 160).

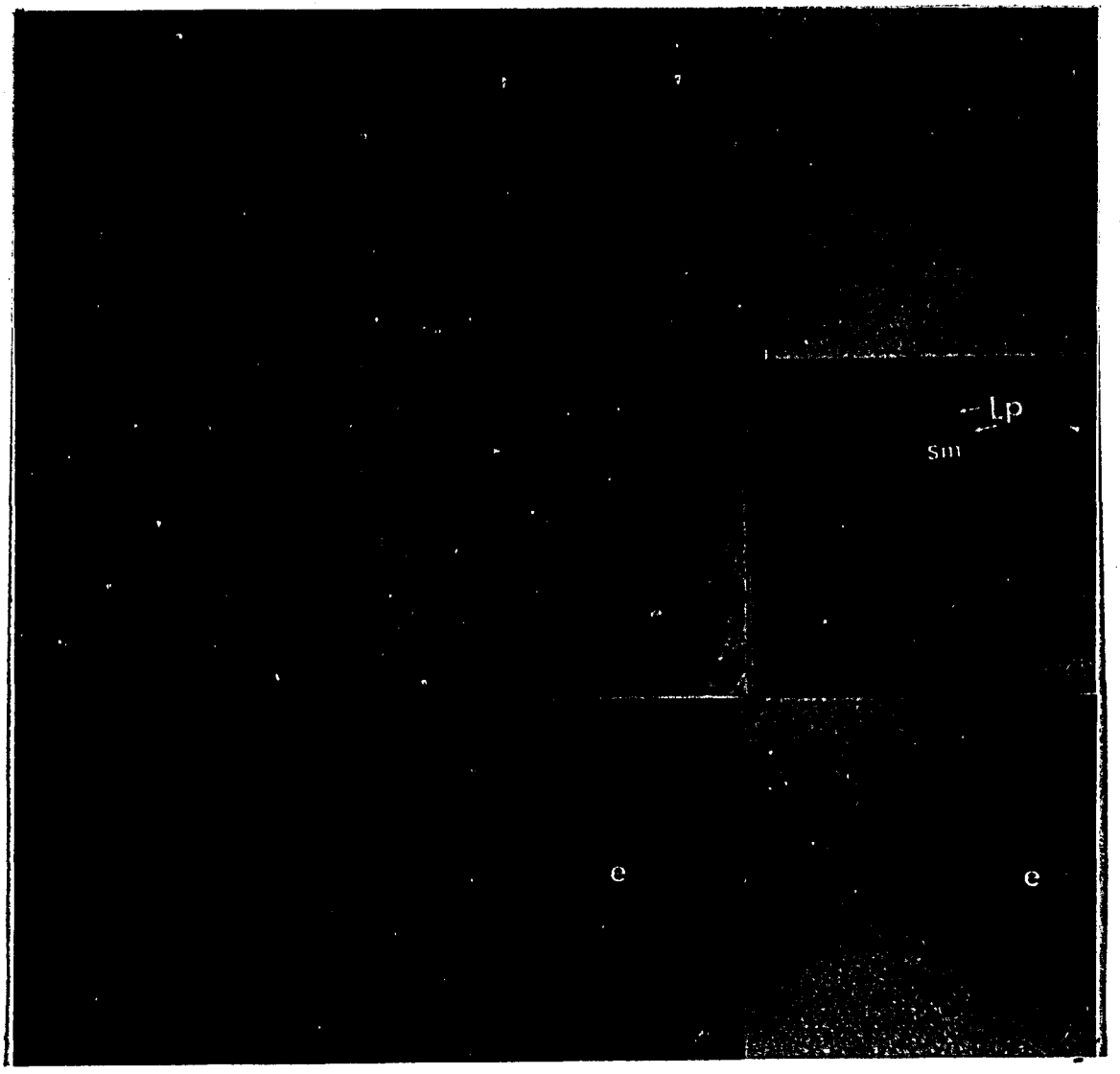

Lp
Sm
e
e

Fig. 28. Longitudinal section showing formation of meristematic cells (mc) for initiation of root primordium (rp) within a callus (x 160).

Fig. 29. Longitudinal section showing initiation of root primordia (rp) from callus (x 160).

Fig. 30. Penetration of roots regenerated from callus (x 160).

Fig. 31. Organization of *in vitro* induced root tip; cortex (c), epidermis (e), procabium (Pr), quiescent center (qc), and root cap (rc) (x 400).

Fig. 32. Longitudinal section of callus showing development of vascular bundle (v) with development of xylem (x) (x 400).

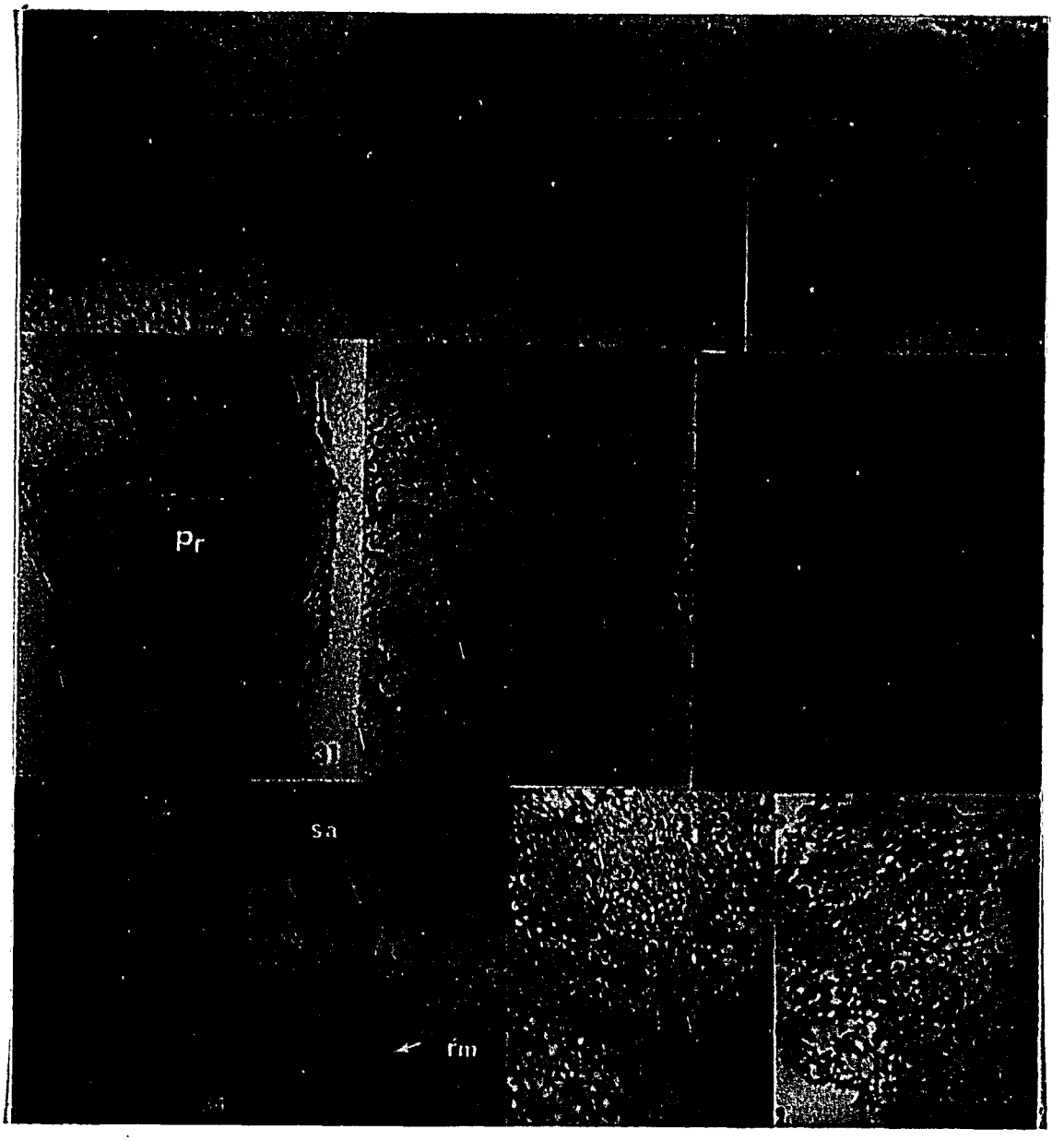
Fig. 33. Longitudinal section showing development of tracheids with annular thickenings in wall (a) (x 400).

Fig. 34. Longitudinal section showing development of tracheids with pitted thickenings in wall (Pi) (x 400).

Fig. 35. Longitudinal section showing initiation of vascular tissue (arrow) connecting a root meristem (rm) and shoot apex (sa) (x 160).

Fig. 36. Cross section showing callus browning in an early stage of growth (x 160).

Fig. 37. Cross section showing callus browning at a later stage of growth. Cells in callus were crushed (cr) (x 160).


pr
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CHAPTER IV

SOMATIC EMBRYOGENESIS IN CELL SUSPENSION CULTURES FROM ROOT CALLUS IN RICE

ABSTRACT

Research was conducted on suspension culture of rice cells. This paper reports on the establishment of cell suspension cultures and embryogenesis in suspension cultures leading to formation of bipolar embryoids. Root tips from seedlings from germinating rice seeds were used as the explant source to initiate callus induction on solid MS medium supplemented with 2,4-D (2 mg/l). Two-week-old calli were dissected into small pieces and placed into liquid MS medium containing 2,4-D (0.1 mg/l) for initiation of a rice suspension culture. The cell suspensions were observed using scanning electron and light microscopes. The cell cultures had two types of cells, small round and large elongated ones. The small round cells showed embryogenic potential and proceeded to embryogenesis. Embryoids at various developmental stages were observed. The most advanced developmental stages of the bipolar embryoids in culture germinated to form roots from coleorhiza.

Abbreviations: MS, Murishage-Skoog medium; 2,4-D, 2,4-Dichlorophenoxyacetic acid; PDA, Potato Dextrose Agar.

Introduction

Regeneration of plants from in vitro culture is a prerequisite for utilization of genetic techniques with somatic cells, e.g. protoplast fusion and genetic engineering, for improvement of crop species [1]. Culture of single cells or protoplasts would be desirable for applications of tissue culture. Successful plant regeneration from suspension or protoplast culture in gramineous species has been demonstrated in millet [2-3] and sugarcane [4]. Although callus induction and plant regeneration through tissue culture has been well documented in rice [5-6], the efforts made to culture rice cell suspension or protoplasts have been limited. Plant regeneration from suspension cultures or protoplast cultures in rice still remains difficult in spite of a few reports in which plant regeneration apparently was achieved from single cells [7-9].

Embryogenesis by plant cells in suspension cultures has been well documented since 1958 when embryogenesis was first discovered in carrot suspension cultures [10]. Somatic embryogenesis in gramineous crops has only been demonstrated recently [3 11].

This paper describes the growth, differentiation, and embryogenesis by rice cells in suspension culture.

Materials and methods

Seeds of rice (Oryza sativa L. cv. Labelle) were dehulled, sterilized in 1.6% sodium hypochlorite solution for 30 min., and transferred to PDA medium for germination. Five-day-old roots were cut about 1 cm from the tip and transferred to MS medium supplemented with 2,4-D (2 mg/l). The plants were incubated at 28°C in the dark for induction of callus.

Two-week-old calli from cultured root tips were dissected into small pieces about 3 mm in diameter and transferred to 30 ml of liquid MS medium supplemented with 2,4-D (0.1 mg/l) in 125 ml Erlenmeyer flasks. Cell suspensions were initiated by culture on a rotary shaker at 125 r min^{-1} under a 16 hr light-8 hr dark regime at 28°C. The resulting cell suspension culture was subcultured weekly by pipetting.

Cell suspensions at various stages of development were fixed overnight in 3% glutaraldehyde, at room temperature, and then washed with 0.1 M phosphate buffer solution. After dehydration through an ethanol series (30, 50, 70, 95, and 100%), specimens were dried in a Polaron Critical Point Drying Apparatus, using liquid CO_2, and coated with gold palladium (200A) using a Polaron sputter coating unit. Observations were made on a Hitachi S-500 scanning electron microscope at 25 KV.

Results

When small pieces of callus derived from rice roots were subcultured in liquid medium on a shaker, cells divided and grew rapidly. The suspension became visible in a few days. When examined with a light microscope, the suspension culture contained a mixture of single cells, groups of cells, and small calli. The single cells and cell masses were suspended in the medium, whereas, the calli fell to the bottom of each flask. A fine suspension was established by weekly transfer of suspended cells to fresh medium.

Suspension cultures from root callus were composed of two basic cell types, that is, round and elongated cells (Fig. 1-2). Round cells were characterized by the presence of a prominent nucleus and they were rich in cytoplasm. These cells tended to be aggregated and showed

competence for embryogenesis in the suspension (Fig. 3). The elongated cells had sparse cytoplasm and appeared large and vacuolated. These cell were single or occasionally grouped together. The morphogenetic competence of such cells, if any, was minimal.

Active embryogenesis was observed among the aggregations of round cells. Embryoid formation at various stages was evident in the suspension cultures. These included the appearance of globular, heart-shaped preembryoids with multicellular suspensor-like structures (Fig. 4-6). Further organization of the preembryoids led to development of bipolar embryoids with a distinct scutellum, coleoptile, and coleorhiza (Fig. 7-8). After prolonged culture of the suspension, the embryoids germinated to produce roots with extensive development of root hairs (Fig. 9-10).

Development of the coleoptile was limited in the present culture system. The most advanced event observed in development of the coleoptile was the formation of a green protuberance (Fig. 7). The surface arrangement of the coleoptile formed in suspension culture was loose and rough in comparison to the compact coleoptile formed in tissue cultured on solid MS medium (Fig. 11-12).

Discussion

Plant regeneration through embryogenesis in cell suspension cultures has been reported for Panicum maximum Jacq. [3] and Saccharum officinarum L. [4]. However, plants were regenerated only from embryogenic callus which was derived from suspension culture and grown on solid medium. The preembryoid stage with notch formation was the most advanced stage in embryogenesis found in their suspension cultures.

Our studies showed that events in early embryogenesis in rice suspension cultures were similar to the process observed in suspension cultures of P. maximum and S. officinarum. Bipolar embryoids were formed in prolonged culture. Further, the embryoid could germinate and produce roots. We attributed the differences in embryogenesis to the different culture systems and plant species.

When the bipolar embryoids germinated, roots from coleorhiza were frequently formed. Development of the coleoptile was inhibited. This may be due to the different requirements for the development of shoots or roots. Recently, it was reported that an increase in the concentration of sucrose and application of cytokinins could enhance germination of embryoids and plant regeneration from suspension cultures [4,9]. It may be possible to regenerate rice plants from suspension culture through embryogenesis by manipulation of culture conditions in further studies.

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Fig. 1; Small round cells found in rice suspension cultures (160 X).

Fig. 2; Large elongated cells in rice suspension cultures (160 X).

Fig. 3; Cell aggregation in rice suspension culture (400 X).

Fig. 4; Development of globular preembryoids in cell suspensions (63 X).

Fig. 5; Development of heart-shaped preembryoids in cell suspensions (400 X).

Fig. 6; Preembryoid in suspension culture with a suspensor-like structure (s) (63 X).

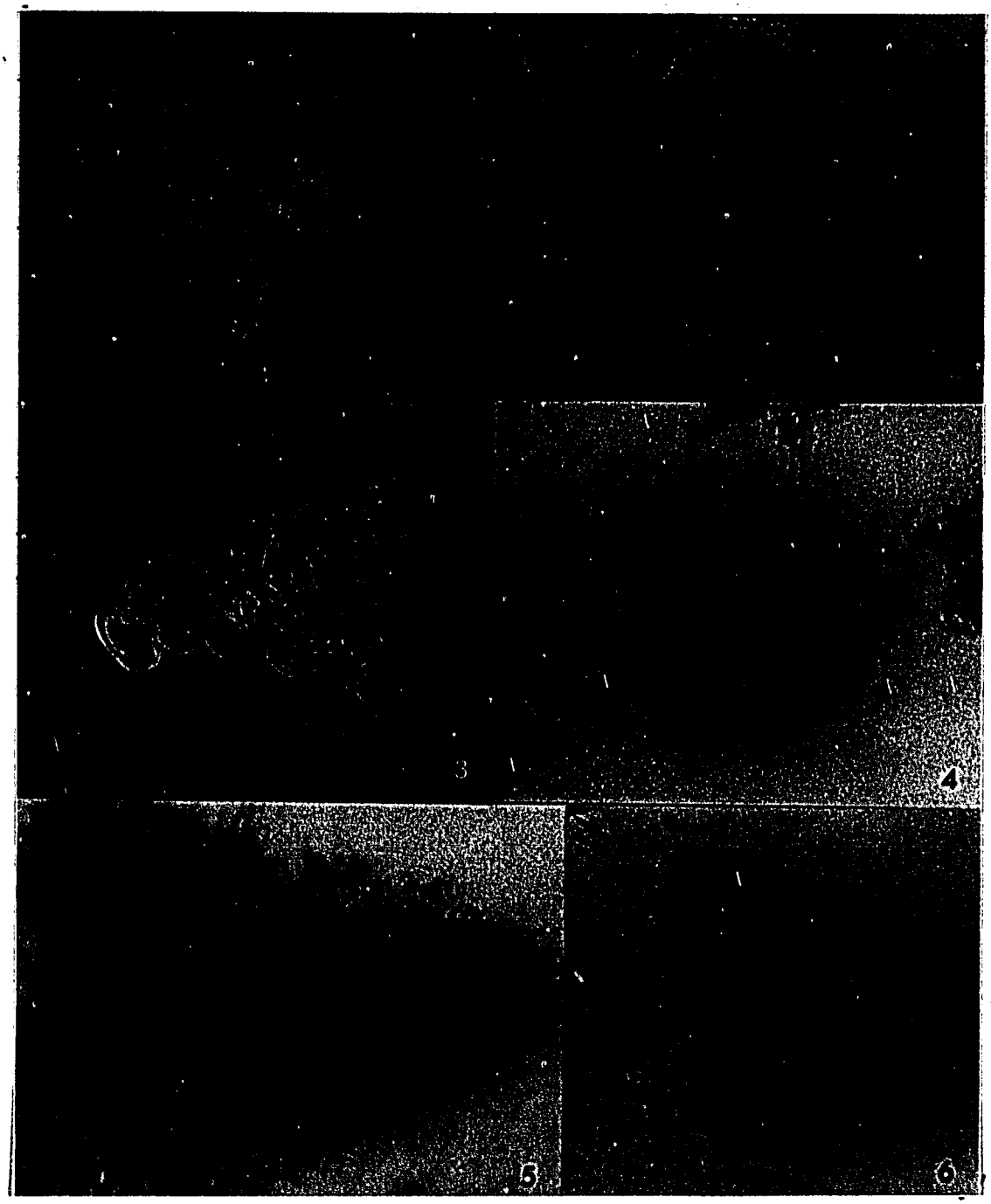
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Fig. 7; Bipolar embryoid from suspension culture (16 X).

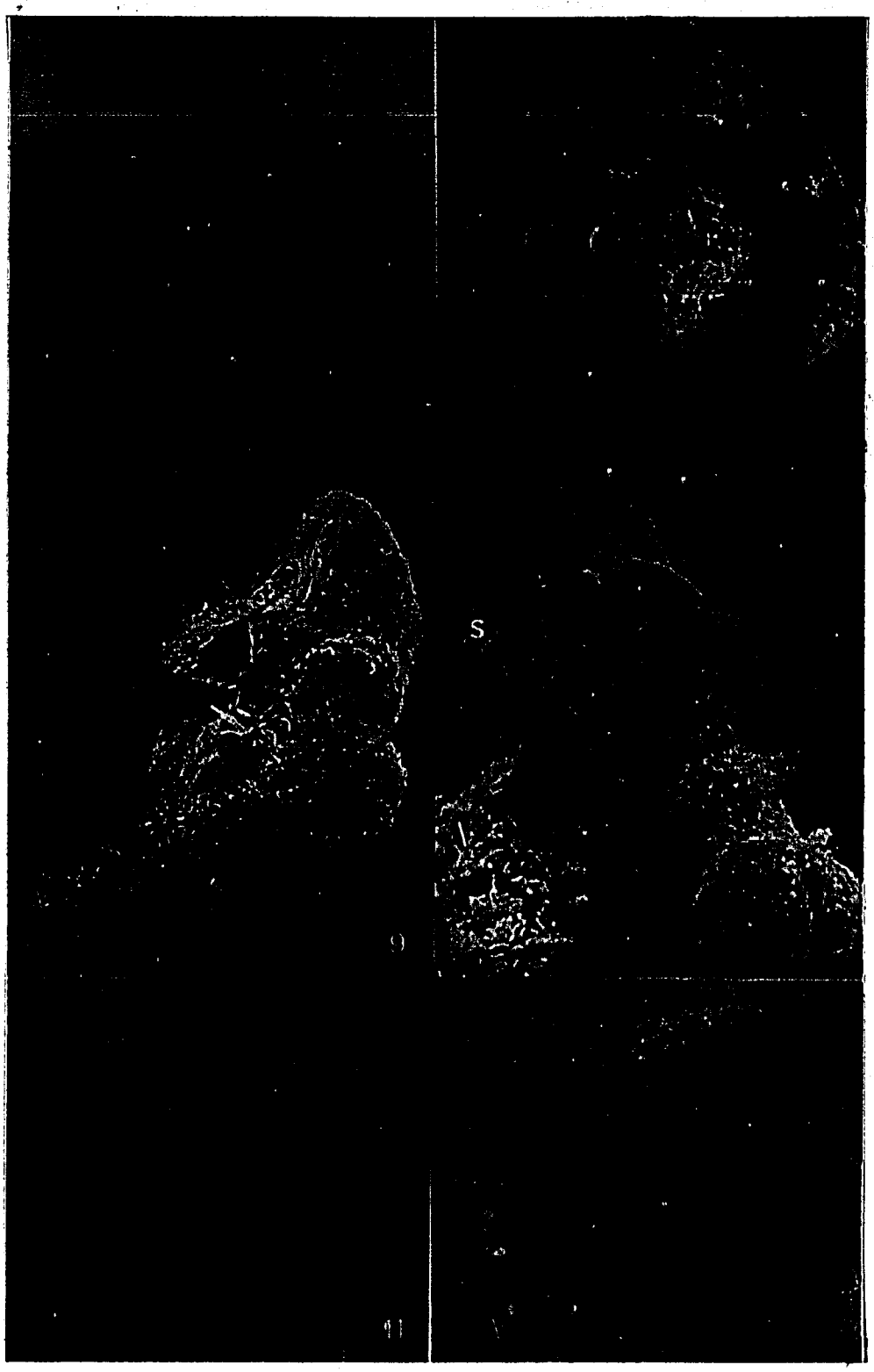
Fig. 8; Scanning electron micrograph of a bipolar embryoid with a scutellum (sc) and coleorhiza (cr) from supension culture (45 X).

Fig. 9; Formation of root (r) from coleorhiza (cr) with coleoptile (cl) (60 X).

Fig. 10; Formation of root (r) with root hair (rh) from coleorhiza (cr) with coleoptile (cl) and scutellum (sc) (60 X).

Fig. 11; Surface of coleoptile (cl) derived from tissue culture on solid medium with scutellum (sc) (150 X).

Fig. 12; Arrangement of surface area of coleoptile (cl) derived from rice suspension culture (175 X).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CHAPTER V

IN VITRO INDUCTION OF GENETIC VARIABILITY IN RICE (*Oryza sativa* L.) SOMACULTURE

ABSTRACT

Somaclones regenerated from the rice cultivars Calrose 76, Mashuri, and Giza 159 were evaluated for variation in morphology and yielding ability to investigate the potential use of somaculture in a rice breeding program. Comparisons between rice somaclones and their parents and within somaclones were made for the presence or absence of awns, plant height, production of albino plants, panicle length, panicle number, panicle weight, seed number, and seed weight. Variation was observed in quantitative and qualitative characteristics. Variation in awn production, plant height, and number of albino plants segregated 3:1. Variation in these characteristics apparently was caused by point mutation of recessive genes in one chromosome of each chromosome pair affected.

Variation in yielding ability was significantly different in progeny lines derived from Calrose 76 and Mashuri. In both cases, R_1 progeny lines with the highest average seed weight per plant yielded 65% higher than lines with the lowest yield values.

INTRODUCTION

Variability derived through plant tissue culture systems has been widely studied and well documented. The detailed discussion of chromosomal alterations given by D'Amata (1978) identified the production of polyploids, anueploids, chromosomal breakage, reciprocal translocations, deletions, and inversions formed during tissue culture as causes of variation. In addition, research on somaclonal variation has demonstrated the occurrence of spontaneous mutations in regenerated plants (Larkin et al., 1981). Somaclonal variation in rice was observed in the first plants recovered from callus tissues in 1968 (Nishi et al.). More extensive studies on somatic genetics in relation to improvement of rice came from Oono (1978), Kucherenko (1979), and Sun (1983). These studies document the occurrence of variations in both quantitative and qualitative characteristics.

The generation of variation is a prerequisite for plant breeding programs. Improvement of rice requires as many germ plasm sources as possible. Plant tissue and cell culture has proven to be a new option for rapidly obtaining increased genetic variability without sophisticated technology. This paper reports on the variation in rice somaclones regenerated from both U.S. and foreign cultivars.

MATERIALS AND METHODS

Seeds of plants regenerated from Calrose 76, Giza 159, and Mashuri were obtained from Dr. Murray Nabors at Colorado State University. The seeds from each regenerated plant were grown in single 20-ft rows at

the Rice Research Station at Crowley, LA. Seeds of each variety were also planted to serve as controls to compare with the progenies of regenerated plants. Single lines derived from individual regenerated plants were designated as the R_1 generation.

At maturity, seeds from each plant were harvested from selected lines and planted in the next generation in single 20-ft rows again. These were designated as the R_2 generation. R_2 progeny lines were compared to the nearest parental line which were inserted every ten R_2 progeny lines as controls.

During the growing seasons, quantitatively and qualitatively inherited characteristics were closely evaluated and/or measured based on the standard evaluation system of the International Rice Research Institute (IRRI 1980). Data for the traits measured were statistically analyzed.

RESULTS AND DISCUSSION

Awn Production

Production of awns in rice is a qualitative trait which can be readily identified. Plants of the cultivar Calrose 76 are typically awnless under normal conditions. Four of 17 R_1 progeny lines from regenerated plants showed segregation for awn production (Table 1). In three of the four lines, the ratio of segregation was 3 awnless plants to 1 awned plant. This was confirmed by Chi-square analysis. The segregation ratio suggested that the trait of awn production was controlled by a single gene. Awnless plants were controlled by a dominant allele, while awned plants were conditioned by a recessive

allele. The occurrence and segregation of awn production among progeny lines from regenerated Calrose 76 plants could have been caused by mutation of a dominant allele into a recessive allele at a single locus. For verification of the Mendelian inheritence, the experiments were carried to the R_2 generation using the pedigree method. Seeds from seven awned and 28 awnless plants from line CR 40 and from 14 awned and 22 awnless plants from line CR 41 were harvested separately and planted in the next generation as R_2 progeny lines (Table 2). All lines derived from awned plants in the R_1 generation produced plants with awns; whereas, the awnless plants in the R_1 generation produced homogenous awnless R_2 lines and lines with a mixture of awnless and awned plants. The ratio of the homogenous and heterogenous lines was 1:2. This suggests that the production of awns was controlled by a single recessive gene. This was an inherited characteristic and awned plants gave rise only to awned progeny lines. The genetic base of the awnless plants was composed of a mixture of homogenous and heterogenous dominant genes. Therefore, segregation occurred in the R_2 progeny lines.

Plant Height

Variation in the mature plant height of R_1 individuals was evident when they were compared with their parent cultivars (Table 3). Although the average plant height of progenies of regenerated plants remained similar to the parent cultivars, the standard deviation for the combined lines were different. The distribution of plant height in the progeny lines of regenerated plants varied more than that of plants of the cultivars with the standard deviation being twice as much among

the progeny lines as that exibited by the cultivars. Comparisons of plants were made between R_1 lines and cultivars and among the R_1 lines. A comparison of plant height between R_1 lines and their cultivar controls is given in Table 4. Most of the lines derived from regenerated plants appeared similar to their parents in mean height. However, the percentage of lines significantly distinct from their parents was 23.5% in Calrose 76, 57.1% for Giza 159, and 21.1% for Mashuri. A detailed comparison showing deviation of progeny lines from the Calrose 76 parent and variation among lines is given in Table 5. Line 38 was taller and lines 40, 48, 61, 62, and 63 were shorter than the cultivar. Line 38 was 12.1% taller than Calrose 76, whereas, line 48 was 13.2% shorter than the cultivar. The shortest plants in line 48 were reduced about 75% in height compared to the cultivars. Among the 17 R_1 progeny lines, there were three lines whose standard deviation reached about or above 15, that is, three times that of the Calrose 76. Heights of individual plants within these lines appeared to be segregating (Table 6). The segregation ratio was 3:1, which suggested that the variation in plant height was controlled by a single gene. Calrose 76 is a semidwarf cultivar. Dwarf plants may have been formed due to the mutation of a dominant height allele into a recessive allele for dwarfing at a single locus through tissue culture. The accuracy of this interpretation could only be determined by crossing one of the tissue culture derived dwarfs with dwarf-types in Calrose 76 controlled by known genes. The frequent occurrence of dwarf plants among progenies of plants regenerated through plant tissue culture appear to be a common phenomenon (Larkin et al., 1984). Similar results reported by Sun, et al., 1983 also indicated that a dwarfing mutation was

controlled by a single recessive gene.

Albino Plants

The occurrence of albino plants during plant tissue culture, and among the progenies of plants derived through tissue culture has been reported (Tamura, 1968)). Albino plants occurred in the R_2 generation of progeny lines from regenerated Mashuri plants grown in the field. Twenty one progeny lines from only one of 19 regenerated plants showed segregation for albino plants. When seed from these 21 lines were germinated in the laboratory, seven lines produced normal green plants and 14 lines produced a mixture of normal and albino plants(Table 7). Chi-square analysis showed that the segregation fitted a 3:1 ratio. These results suggested a single gene inheritance for this character. The lines showing normal green plants were derived from homozygous plants and the lines with a mixture of green and albino plants apparently were the progenies of heterozygous plants. Albino plants occur more frequently in rice anther culture than somaculture (Devreux, 1970; Wang et al., 1977). Observation of cells of plants produced by anther culture through electron microscopy showed that albino rice plants had proplastids deficient in lamellae structure when compared to normal chloroplasts (Sun et al., 1974). Biochemical analysis showed that the fraction I protein, 23S and 16S RNA of plastid ribosomes were absent in albino rice plants. These proteins were essential for normal development of chloroplasts (Sun et al., 1979). Plastid ribosome and fraction I protein are coded by both chloroplast and nucleic DNA (Thomas and Tewari, 1974; Bourque and Widman, 1973; Criddle et al., 1970). Any disturbances in plastid and nucleic DNA could cause a

deficiency of plastid ribosomes leading to the occurrence of albino plants (Borner et al., 1972; Sprey, 1971; Toyama, 1972). Our data suggests that mutation of a nuclear gene controlling the development of chloroplasts resulted in the production of albino plants. This mutation occurred at a single locus on one of a pair of previously homozygous chromosomes.

Components of Yield

A primary evaluation was made on yielding ability among progeny lines of plants regenerated from Calrose 76 (Table 8) and Mashuri (Table 9). There were significant differences among the progeny lines for this characteristic. Comparisons were only made among somaclonal lines as they were planted with the same number of seeds per row, whereas, the cultivar control plots had a higher number of seeds per plot. Line 39 showed the best yielding ability among the progeny lines of plants regenerated from Calrose 76 (Table 8). This line had an increase of 62.7% over the mean seed weight per plant of line number 40, the lowest yielding line. Contributing to this increase in seed weight were positive increases in panicle weight, panicle number, and panicle length. Line 40 had low values for all of these yield components. Line number 39 had an increase in panicle weight of 88.3%, in panicle number of 50.7%, and in panicle length of 17.9% over line number 40. Among the progeny lines of plants regenerated from Mashuri (Table 9), the average seed production of individual progeny in line 53 appeared lowest. It also showed low values in seed number, panicle weight, and panicle numbers. Line 55 showed the highest mean seed weight production by individual plants with 64.5% more than line 53 and

24% more than the average for all lines. Variation in seed weight has previously been reported among progeny lines derived from anther culture of Calrose 76 (Schaeffer, 1984). Among 25 doubled haploid lines, four lines gave significantly higher seed weights than the cultivar control. Our research also showed wide variation ocurring in yielding ability in progeny derived from somaculture. The significance of this study lies in the demonstration of variation derived through *in vitro* techniques. This could provide a rapid way to create variation in commercial cultivars or breeding lines. Further studies of somaclonal variation in rice are in progress.

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Table 1. Awn production in the R_1 generation of lines from plants produced through somaculture of Calrose 76.

Line number	No. of plants	No. of awned plants	No. of awnless plants	Expected ratio	X^2	P
CR-40	44	10	34	1:3	0.129	0.50-0.75
CR-41	38	15	23	1:3	3.332	0.05-0.10
CR-61	8	2	6	1:3	ER = OR[+]	
CR-62	8	2	6	1:3	ER = OR[+]	

[+] The expected ratio equaled the observed ratio.

Table 2. Inheritance of awnless progenies of plants produced through somaculture of Calrose 76.

Line number	No. of awnless plants in the R_1 generation	R_2 generation: No. of awned plants	No. of awnless plants	Expected ratio	X^2	P
CR-40	28	7	21	1:2	0.873	0.25-0.50
CR-41	22	7	15	1:2	0.023	0.75-0.90

Table 3. A comparison of plant heights among R_1 progenies and their parents.

Rice cultivar or line	Minimum height (cm)	Maximum height (cm)	Mean	Standard deviation
Giza 159	89.0	98.0	93.9	2.63
R_1 lines	86.5	117.0	102.7	6.00
Calrose 76	80.0	100.6	92.1	4.37
R_1 lines	22.0	107.8	90.3	10.98
Mashuri	88.0	100.0	93.0	3.56
R_1 lines	70.0	117.0	95.0	7.43

Table 4. A comparison of plant heights among R_1 lines and their parents.

Rice cultivars	No. of lines		
	Taller	Equal	Shorter
Calrose 76	2	13	2
Giza 159	4	3	-
Mashuri	1	15	3

Table 5. Variation in plant heights among R_1 lines from plants regenerated from Calrose 76.

Line no.	No. of plants	Minimum height (cm)	Maximum height (cm)	Mean height (cm)	Standard deviation	F
CALROSE 76	50	80.0	100.6	90.1	4.4	12.35**
CR-37	32	71.0	107.8	98.5	6.0	
CR-38	28	93.1	106.5	101.0	3.6	
CR-39	36	90.2	106.2	99.2	3.3	
CR-40	40	39.5	99.1	85.3	14.8	
CR-41	36	71.0	104.2	91.4	9.7	
CR-42	28	83.3	95.3	90.2	3.5	
CR-43	26	80.4	94.5	88.5	3.4	
CR-44	20	80.5	95.4	90.3	4.3	
CR-45	17	70.7	95.0	87.1	5.4	
CR-46	19	66.2	95.2	86.0	6.2	
CR-47	24	80.0	98.2	88.2	4.4	
CR-48	29	22.0	97.0	78.2	17.7	
CR-60	9	82.2	99.5	90.6	5.2	
CR-61	7	67.4	98.0	87.9	11.8	
CR-62	7	63.5	94.3	84.0	10.3	
CR-63	5	43.0	90.4	75.8	21.1	
CR-64	5	82.0	90.0	85.7	2.9	

** Significant at the P=0.01 level.

Table 6. Segregation of plant height in the R_1 generation of three Calrose 76 somaclones.

Line no.	No. of Plants	Plant size		Expected ratio	X^2	P
		Normal	Dwarf			
CR-40	40	30	10	3:1	ER = OR[+]	
CR-48	29	20	9	3:1	0.563	0.25-0.50
CR-63	5	4	1	3:1	0.067	0.75-0.90

[+] The expected ratio equaled the observed ratio.

Table 7. Segregation of albino plants in R_2 lines from one somaclone of Mashuri rice.

R_2 lines[+]	No. of seeds	No. of seeds germinated	Plant color		Expected ratio	X^2	P
			green	albino			
1	50	47	47	0			
2	50	49	38	11	3:1	0.17	0.50-0.75
3	50	48	36	12	3:1	ER = OR[++]	
4	50	48	35	13	3:1	0.11	0.50-0.75
5	50	49	49	0			
6	50	50	37	13	3:1	0.01	0.75-0.90
7	50	48	40	8	3:1	1.78	0.10-0.25
8	50	48	34	14	3:1	0.35	0.50-0.75
9	50	48	38	10	3:1	0.35	0.50-0.75
10	50	48	36	12	3:1	ER = OR[+]	
11	50	49	49	0			
12	50	49	37	12	3:1	0.01	0.75-0.90
13	25	24	24	0			
14	50	49	33	16	3:1	1.53	0.10-0.25
15	20	20	14	6	3:1	0.27	0.50-0.75
16	50	50	40	10	3:1	0.56	0.25-0.50
17	50	48	34	14	3:1	0.35	0.50-0.75
18	50	49	49	0			
19	20	20	20	0			
20	50	49	34	15	3:1	0.82	0.25-0.50
21	50	49	49	0			

[+] Each line was derived from a single R_1 plant.

[++] The expected ratio equaled the observed ratio.

Table 8. Variation in yield components among rice somaclones derived from Calrose 76.

Line no.	Mean seed weight per plant (g)	SD+	Mean seed number per plant	SD	Mean panicle weight per plant (g)	SD	Mean panicle number per plant	SD	Mean panicle length (cm)	SD
CR-37	14.0	7.4	489.8	259.7	15.0	7.7	-	-	-	-
CR-38	17.0	6.6	459.5	250.3	18.1	7.0	-	-	-	-
CR-39	19.2	7.6	652.8	290.8	20.9	7.9	11.0	4.3	18.4	0.8
CR-40	10.5	7.1	427.3	278.5	11.1	7.4	7.7	3.9	15.6	2.5
CR-41	11.8	7.3	478.0	287.6	12.4	8.1	7.3	4.0	16.4	2.4
CR-42	15.0	9.1	578.3	292.7	14.9	7.4	9.1	4.3	16.7	0.7
CR-43	15.0	7.4	681.2	333.0	15.8	7.7	10.6	5.0	16.9	1.6
CR-44	15.4	6.6	733.2	362.4	16.5	7.0	10.0	4.0	17.8	1.1
CR-45	15.0	6.3	677.2	258.8	16.0	6.6	10.0	3.3	16.9	0.9
CR-46	13.7	6.7	506.7	241.3	14.5	6.9	8.9	3.9	17.1	1.0
CR-47	13.7	5.6	550.2	214.7	14.5	5.8	9.7	3.4	16.9	1.0
CR-48	14.4	10.6	648.6	459.4	15.3	10.8	8.5	5.0	17.2	1.1
LSD (P=0.05)	4.2		165.1		4.2		2.3		0.9	

+ Standard deviation

Table 9. Variation in yielding ability of R_1 somaclonal lines derived from Mashuri rice.

Line no.	Mean seed weight (g)	SD	Mean seed number per plant	SD	Mean panicle weight (g)	SD	Mean panicle number per plant	SD	Mean panicle length (cm)	SD
M-51	12.9	6.4	847.7	456.9	13.6	7.1	13.7	6.5	17.5	1.3
M-52	13.9	8.6	938.2	575.3	16.4	11.2	14.9	8.4	18.6	1.4
M-53	11.0	5.7	721.7	365.4	12.3	6.5	10.6	5.0	17.9	3.1
M-54	11.7	9.8	809.1	631.0	14.4	11.3	13.0	8.2	18.1	1.5
M-55	18.1	9.9	1084.1	540.5	18.6	10.7	16.0	7.5	17.7	3.0
M-56	16.7	7.1	1149.7	470.0	17.5	6.9	16.9	6.4	16.9	2.0
M-57	16.2	10.4	1084.8	668.5	15.9	9.7	14.1	7.6	17.2	1.4
M-58	17.3	9.7	1300.0	766.5	18.7	11.3	16.6	8.9	17.9	1.0
M-59	13.3	9.7	935.4	636.7	16.0	13.3	13.3	9.0	18.2	2.0
LSD (P=0.05)	4.8		165.1		5.6		4.2		1.2	

CHAPTER VI

SOMACLONAL VARIATION FOR RICE IMPROVEMENT

ABSTRACT

Two hundred somaclones from the rice cultivar Labelle and one hundred somaclones from the cultivar Lemont were selected for evaluation in the field in 1985 for variation in morphology and yielding ability. An additional 358 lines from Labelle and 218 lines from Lemont were tested in the field for changes in resistance to rice sheath blight. Variations were observed in the following characters: leaf blade angle, flag leaf angle, culm angle, leaf color, leaf pubescence, leaf senescence, culm strength, resistance to sheath blight, panicle type, panicle exsertion, sterility, seedling height, mature plant height, tiller number, leaf blade length, leaf blade width, days to heading, panicle length, panicle number, panicle weight, 100-seed weight, and average yield per line. Chi-square tests for variation in segregation of albino plants, pseudo-blast leaf color, plant height, days to heading, and sterility indicated that these characters were controlled by a single recessive gene. Somaclonal line number 726 from Lemont had a statistically significant 12% increase in yield over that of the cultivar Lemont.

INTRODUCTION

Heritable phenotypic differences are the basis of any plant breeding program. Although conventional rice breeding efforts have provided cultivars with significantly increased production in the past few years, continuous improvement of cultivars requires modification of one or a few undesirable characters with minimum disturbance of the overall genetic combination that gives high yield and good agronomic characteristics. Substantial evidence has accumulated that much of the variation generated during somaculture is heritable and could be used for crop improvement and genetic studies (Larkin, et al, 1981).

Variation in rice somaclones and the inheritance of this variation has been studied by several researchers (Oono, 1978, Kucherenko, 1979, Sun, et al, 1983). The traits observed in those studies included leaf color, plant height , tillering, sterility, and some yield components. Somaclones from the cultivars Labelle and Lemont, which are long-grain commercial varieties popular in the Southern United States, were produced in our laboratory. The objective of this study was to evaluate the potential for variation in morphology, yield potential, and disease resistance of somaclones derived from these two cultivars.

MATERIALS AND METHODS

Genotypes used in this study were somaclones derived from the Oryza sativa L. cultivars Labelle and Lemont. Calli were initiated from nodes, immature panicles, and mature panicles using Murashige-Skoog (MS) medium containing 4 mg/l 2,4-D. Plants were regenerated from calli on

MS medium supplemented with 0.5 mg/1 IAA and 0.8 mg/1 BA. The details of the culture procedures and media for rice tissue culture were previously reported (Jun, 1986) and are given in detail in Appendices 2, 3, and 4.

Regenerated plants with large root systems were transplanted from petri dishes into soil in 8-inch black plastic pots placed in a greenhouse. Pots had a 2:1:1 mixture of soil, peat, and sand. Each pot contained 2 or 3 regenerated plants. Somaclones were shaded with brown paper for 5 to 10 days after transplanting and were watered as needed with deionized water. Plants were fertilized with sufficient liquid fertilizer for normal growth. As panicles began to emerge, a waxed paper bag was placed on each panicle for the prevention of outcrossing. At maturity, seeds from each plant were collected for growing the next generation (R_1). Variation in certain characters, such as, awn production, apiculus color, sterility, and seed size in the R_0 generation were recorded.

About 50 seeds from each of 200 regenerated plants from Labelle and 100 somaclones from Lemont were planted in separate pots for germination in a greenhouse. Seeds of the cultivars Labelle and Lemont were also planted. At the 4 or 5 leaf stage, the R_1 plants were transplanted to the field in May 1985 at the Rice Research Station at Crowley, LA. A randomized complete block design with three replications of 15 transplants per line was used in this test. Each line consisted of 45 progenies of the selfed somaclones. The plant to plant spacing within lines was 20 cm and row to row spacing, with one row per line, was 30 cm. A row of the parent cultivar was grown every 10 rows as the control.

Twenty four characters were observed and /or measured during and after the growing season. All plants of each line and control row were quantitatively measured for the following characters: seedling height (8 weeks after planting), length of the penultimate leaf at maturity, width of the penultimate leaf at maturity, mature plant height, panicle length, panicle number, panicle weight, 100 seed weight, and yield. Individual plants of each R_1 line and the check rows were observed visually and assigned scores according to the Standard Evaluation System of the International Rice Research Institute (IRRI 1981) for the following characteristics: blade color, basal leaf sheath color, flag leaf angle, ligule color, collar color, tiller number, tiller angle, culm internode color, culm strength, days to heading (emergence of 50% of the panicles per plant 1 cm above the flag leaf collar), panicle type, panicle exsertion, awn production, leaf blade pubescence, sterility, and leaf senescence.

An additional 358 somaclonal lines from Labelle and 218 somaclonal lines from Lemont were drill-seeded as single-row plots to screen for resistance to rice sheath blight caused by Rhizocotonia solani Kuhn. One row of the parental cultivar was included every 10 lines as a control. Rows were 1.3 M long and seeded 24 cm apart with lines planted in the order of the somaclone numbers. R. solani isolate No. LR 172 was grown on a sterilized mixture of rice grains and rice hulls (2:1). At the late vegetative stage of growth, the plots were inoculated with 25 ml/row of the R. solani inoculum. All plots were rated at maturity for sheath blight using a 0 to 9 scale with 0 equal to no disease and 9 equal to all plants dead at maturity (Hoff, et al., 1977).

All quantitative data were statistically analyzed using SAS

computer programs. Data values for the traits measured were statistically compared to the parent cultivar from which each line was derived to determine if the observed variation among the traits were significant.

RESULTS AND DISCUSSION

Variation in the R_0 Generation

Labelle and Lemont plants are awnless. Some somaclones from these varieties possessed awns (Table 1). About 3% of the regenerated plants from Labelle and 2% from Lemont had awns. The awned plants all had large spikelets. Apiculus color of some awned plants appeared white in contrast to the normally purple-red apiculus of Labelle or pinkish-red apiculus of Lemont. Seed production from these plants was poor with a range from zero to a few seeds.

Observations on the sterility of regenerated rice plants were made on the basis of two classifications, that is, fertile (plants producing grain) and sterile (plants not producing grain). The frequency of sterility in the R_0 generation was about 5% for both Labelle and Lemont. Other variants observed in R_0 plants included albinism, chlorophyll deficiency, variation in height, and an occasional plant was produced with reddish-purple lower sheaths.

Variation in the R_1 Generation

Plant type

Plant type is an important morphological character in relation to rice breeding. Normal plants of Labelle had a leaf blade angle of about 45°, horizontal extension of the flag leaf, and a culm angle of 45° or less. Normal plants of Lemont had upright leaf blades and flag leaves with a culm angle of about 45°. Somaclonal lines of plants regenerated from Labelle and Lemont showed a range in these three characters (Table 2). Most lines from regenerated plants resembled their parental cultivars in these characteristics. A few lines deviated greatly from their parental cultivars. Plant culms could have either a wide angle or be more erect. Leaf blade angle could be erect, drooping or descending.

Leaf Blade Characters

Variations in leaf blade color were observed. Two plants from separate progeny lines originated from Lemont and two plants from Labelle showed chlorophyll deficiency. These plants appeared less vigorous and had slow growth and poor tillering. Two additional progeny plants from Lemont were variegated. These plants were found in lines from regenerated plants with normal green leaves. The frequency of regeneration of plants with chloropyll deficiency was 2% and 1% for variegation.

A few somaclonal lines showed segregation for albino plants (Table 3). The albino plants were easily detected from two R_1 lines among 200 progeny lines after their germination. A Chi-square test of the segregation ratios in these two lines showed that the pattern of segregation fit the ratio of 3:1 for line number 269 and 9:7 for line

number 114. The albinism was inherited as a trait controlled by one gene locus in which albinism was recessive to the colored allele in line 269. Albinism was the result of a recessive gene at either of two loci that controlled the trait in line 114 (Table 3).

At the early vegetative stage, a somaclonal line showed symptoms similar to the lesions produced after infection by Pyricularia oryzae Cav. The lesions appeared on the surface of leaf blades having a purple color. P. oryzae could not be isolated or detected. This character was called pseudo-blast. Genetic analysis showed quite clearly that the pseudo-blast trait was controlled by one gene locus where purple leaf color and lesions were recessive to the normal leaf allele (Table 4).

At the late vegetative stage, flag leaves of somaclonal progenies from two regenerated plants showed yellowing or browning of their leaf tips. These plants exhibited abnormal leaf rolling. A Chi-square test of the segregation exhibited by these line suggested that in line number 304 the brown-tip character was inherited as a single recessive gene. In the other line this character was inherited as two independent genes (Table 5). The observed phenomenon which showed two different segregation ratios for leaf-tip browning could be expected if we assumed inheritance of the character was governed by two independent genes which were located on separate chromosomes. The trait could be expressed only when the two genes were in the homozygous recessive condition. If one dominant allele of either of the two genes was present, the normal green leaf resulted. Thus, donor plants were homozygous dominant and mutation from dominance to recessive occurred during the plant tissue culture cycle.

Leaf blade surfaces of the cultivars Lemont and Labelle were glabrous. Five somaclonal progenies from three plants regenerated from Labelle and five somaclonal progenies from four regenerated plants from Lemont were pubescent. The plants with pubescence were morphologically different from the glabrous somaclonal progenies and from each other. Some plants were characterized by late maturity, medium grain, and green apiculus color; while others showed large grains with awn production. The frequency of generation of pubescent variants from rice somaculture was 1.5% for Labelle and 4% for Lemont.

Senescing leaves were observed at the time of harvest for their retention of green color (Table 6). Most somaclonal lines resembled their cultivars, with leaves below the flag leaf becoming yellow or withered and the flag leaf turning to yellow maturity. One somaclonal line showed slow senescence and the flag leaf and penultimate leaf retained the normal green color at maturity. A few lines appeared to senescent faster than the cultivars and their leaves were completely dead when the grains were fully ripened.

Panicle Characters

Variation was also observed in panicle type and exsertion of the panicle from the flag leaf sheath (Table 7). For evaluation of panicle type, the angle of primary branches and spikelet density were considered. Most somaclonal lines were similar in panicle characteristics to the cultivar from which they were regenerated. Two cultivars had intermediate extension of primary branches with relatively heavy spikelet density. However, there were a few lines that varied by

having either compact or open panicle type.

Grading for panicle exsertion was mainly based on the position of the panicle base in relation to the flag leaf sheath collar. The majority of plants from both parent cultivars and the somaclonal lines were moderately well exserted. The position of the basal node of the panicle was located from about 1 cm above to even with the flag leaf collar. A few somaclonal lines had panicles that were exserted about 3 cm above the flag leaf collar.

Culm Strength

The degree of culm strength is positively correlated to lodging resistance. In 1985, two hurricanes passed over the plot area when Labelle and its somaclonal lines were approaching maturity. Culm strength of the cultivar Labelle was weak and severe lodging resulted from the hurricane winds. Most somaclonal lines manifested similar or poorer response to the winds. Plants in most lines were completely lodged. However, two lines appeared to have lodging resistance based on records from three replications (Table 6).

Sterility

Individual plants in six out of 198 somaclonal lines derived from Labelle showed sterility. The sterility ranged from partial to complete sterility. When the number of fertile plants was compared to the number of sterile plants, the segregation ratio appeared to be 3:1 for four of the six lines. This was confirmed by Chi-square test (Table 7). Among

these six somaclonal lines, the lines numbered 770 and 771 originated from the same callus and sterile plants from these two lines appeared dwarf, slow growing, and never reached the heading stage. These plants may have been photoperiod sensitive and not sterile.

Quantitative Characteristics

Ten characteristics were quantitatively measured and statistically analyzed. Variation in all ten characteristics was greater in the somaclonal populations than in the parental cultivars. The ranges of these characteristics were much wider for the lines. The standard deviation values for all quantitatively measured characteristics were higher than those obtained from the cultivar although the differences were small because a large population of somaclonal progenies was analyzed in comparison to the relatively small cultivar population.

Height of Labelle and Lemont plants and their somaclonal lines was measured 8 weeks after planting and at maturity. Although mean values of seedling height and plant height between somaclonal populations and their parents were very close, more than 50% of the somaclonal lines individually showed significant differences from their parents, being either taller or shorter than the parent cultivar (Tables 8, 9, 10). The height of the shortest line was reduced by 14.3% and tallest line was increased by 8.0%. Comparison was further made to examine development of the lines which showed either significantly higher or shorter plants than the parent cultivar in the vegetative stage (Table 11). There was a significant correlation among heights of variants in the seedling stage and mature plant height. Eleven of 12 somaclonal lines which were significantly taller than the parent cultivar at the

vegetative stage remained significantly taller as mature plants. Seven of 12 somaclonal lines which were shorter than the parent at the vegetative stage remained significantly shorter. Measurable differences in plant height could be determined before plants reached the reproductive stage. Selection for dwarf and semidwarf plants could be made at the vegetative stages of growth. Observations were also made on the amount of variation within a line. It was found that plant height in Lemont lines 417 and 565 could be divided into two groups. About one-third of the plants within line 417 were semidwarf with their morphology greatly changed from the cultivar. These plants had thin culms, narrow leaves, and they produced few seeds. Dwarf plants in line 565 were different from the cultivar and from the dwarfs in line 417. These dwarfs averaged only 40 cm in height with dark green leaves, reduced blade length, and an increase in blade width and thickness. These plants produced small round grains (Table 4). The Chi-square analysis for these two lines suggested that both types of dwarfing were controlled by single recessive genes. Segregation from normal plants could have resulted from spontaneous mutations at different loci.

The requirement for a certain growth period before heading is a characteristic of a particular genotype, although this character is affected by environmental conditions. In our experiments, the days necessary for growth and development before heading were about 78 days for Labelle and 88 days for Lemont. The days to heading for individual plants in somaclonal lines ranged from 71 to 109 in Labelle somaclones and from 71 to 117 days for Lemont somaclones. About 21% of the Lemont somaclonal lines and 51.5% of the Labelle lines had significant variation in the time from planting to heading. Lines with variation in

days to heading were about equal between either longer or shorter growth periods with Lemont somaclonal lines. In the Labelle somaclonal lines, about three times as many lines had a shorter period to heading as compared to these with a longer period to heading than the cultivars.

Variations in time to heading within the lines which appeared to be segregating were recorded (Table 12). There were five lines from Lemont in which about one-third of the individuals required 98 days or more before heading. These individuals were grouped as late heading. The Chi-square test indicated that the observed segregation ratio fit a Mendelian inheritance pattern based on a single gene for late maturing.

Tillering ability is a quantitative character. Under certain conditions, it is positively correlated with yield. Tiller number was measured among 100 Labelle somaclonal lines. The mean tiller number for the Labelle cultivar was 20.8. The mean tiller number for somaclonal lines ranged from a 26.4% reduction to a 42.8% increase compared to Labelle. The highest tiller number for an individual plant in a somaclonal line was 57. This value was about one and half times more than the mean value obtained by the Labelle cultivar and also 35.7% greater than the highest tiller number reached by any individual Labelle plant in the control rows. All plants in the test were space planted to ensure sufficient room for expression of their maximum inherent ability to tiller.

Significant variation occurred in leaf blade length and width among the somaclonal lines from both cultivars. Thirty percent and 44% of the Labelle somaclonal lines were significantly different from the parent cultivar in leaf blade length and width, respectively. The highest mean values for somaclonal lines showed increases of 14% in leaf blade length

and 23% in width.

Yield is a quantitatively controlled characteristic and can be considered a summary of the performance of an individual plant or plant populations throughout the growing season. Many factors contribute to yield including panicle and seed characters. Factors contributing to yield include panicle length, number, weight, and 100-seed weight. These factors and mean yield by line for each somaclone derived from Lemont were measured. The mean value for panicle length for the somaclonal population was 13.5% higher than the Lemont control. The highest value for panicle length of an individual plant in the somaclonal population was 23% higher than the cultivar mean. This plant had a 15.4% greater mean panicle length than the highest value obtained for individual plants in the Lemont control. Eighty one percent of the somaclonal lines had a significantly greater panicle length than the parental mean value. Only two of 100 somaclonal lines were significantly shorter than the mean value for panicle length of Lemont with the greatest reduction being 4.5%. In contrast, the mean values for 100-seed-weight were generally lower in the somaclonal population when compared to the value obtained for the Lemont control. Ninety-five percent of the somaclonal lines had significantly lower 100-seed weights than the parental mean value. However, seeds produced by the Labelle somaclonal line 215 showed a large increase in seed size. The mean 100-seed weight of this line was 2.6 g and the highest 100-seed weight of an individual plant in this line reached 2.9 g. The mean 100-seed weight of the Labelle cultivar was 2.0 g. The yield of this line was not measured because of the hurricane. Seed were collected for a future yield comparison with the Labelle control.

About fifty percent of the Lemont somaclonal lines were distinctly varied in panicle number from their parent. Thirteen percent of the lines had an increase in panicle number. The highest number of panicles produced by an individual plant in the somaclonal population showed about a 90% increase over the mean panicle number of the control plants and about a 16% increase in comparison with the highest panicle number produced by an individual plant in the cultivar control.

Variation in panicle weight showed a highly significant correlation with variation in yield by line with r=0.86 (Table 13). Each of 10 lines which showed either higher or lower panicle weight than the Lemont control were also compared for yield. Yield was generally lower in the somaclonal population than in the parent population. There was a 7% overall reduction in both panicle weight and yield when somaclonal lines were compared to Lemont. However, the individual plant in the somaclonal population with the highest increase in panicle weight or yield over the cultivar mean increased by 124% and 113.4%, respectively. There was a 24% increase in panicle weight and a 13% increase in yield by this plant over the highest value for individual plants in the Lemont control. Among 100 somaclonal lines, 17 lines had a higher yield than the cultivar control. Five lines had at least a 5% increase in yield compared to the control. The lines 726 and 196 were significant higher in yield than the Lemont cultivar with P = 0.05. Line 726 had a mean yield 12.4%. higher than the cultivar.

The frequency of variation in quantitatively measured and visually evaluated characters among 50 somaclonal lines from Lemont were estimated based on the classification of lines differing significantly from the control as being variants (Table 14). Much greater variation

was associated with quantitatively measured characters. Every line showed simultaneous variations in at least two characters. In general, the somaclonal lines from Lemont showed variation in four quantitatively measured characters. There were two lines which varied in all seven of the characters measured. Visually evaluated characters were much less variable. Most of the lines showed variation in a single character. One line showed variation in four visually evaluated characters.

Frequency of occurrance of the somaclonal lines from Lemont which carried mutant genes for which some estimate of the Mendelian inheritance could be estimated are listed in Table 15.

2,4-D Effects

Effects of the concentration of 2,4-D in the callus induction medium on variability were evaluated based on seven quantitative characters. It appeared that there was a pattern in the occurrence of variability in plants regenerated from calli produced on MS media having different concentrations of 2,4-D (Table 16). The occurrence of variability among the progenies of somaclones regenerated from callus induced on the MS medium supplemented with 1 mg 2,4-D had the highest standard deviation value in all the seven measured characters. Mean values of these progenies were lowest in all measured characters except seedling height. The progenies of the regenerated plants from callus induced on the MS medium containing 4 mg 2,4-D showed highest values in seedling height, panicle weight, yield by line, and 100-seed weight. The effects of concentration of 2,4-D on occurrence of genetic variation in somaclones regenerated from barley tissue were reported by Deambrogio

and Dale (1980). Progeny of plants regenerated from barley exposed to a range of 2,4-D from 4.5 to 18 M showed variation only among plants regenerated on medium with 2,4-D at a concentration of 18 M. Variation was in albinism, leaf shape, tiller number, and fertility. The relationship between 2,4-D and frequency of occurrence of variability among somaclones is not understood. The present evidence suggests that an increase in variability is associated with the presence of 2,4-D at certain levels.

Resistance to Rice Sheath Blight

Resistance to rice sheath blight was evaluated among 576 progeny lines of plants regenerated from the cultivars Labelle and Lemont (Table 17). The labelle and Lemont cultivars were very susceptible to rice sheath blight. Most of the progeny lines from regenerated plants remained susceptible to R. solani. Two somaclonal lines from Lemont showed reduced susceptability to rice sheath blight with a rating of 5. Two somaclonal lines out of 358 regenerated plants from Labelle also appeared resistant to rice sheath blight with a 3 rating. The cultivar rows had ratings of 7, 8, and 9. Plants of the resistant Labelle lines had increased stem and leaf blade thickness and produced large seeds with awns. The characters were similar to those described for polyploid or aneuploid plants. The chromosome number of plants from these lines has not been studied. Evaluation of Labelle somaclonal lines for resistance to rice sheath blight in the field led to the finding of the two progeny lines apparently resistant to rice sheath blight. Although these two lines need to be further tested for their resistance, these

results suggest that plants regenerated through somaculture could vary in their disease resistance. Such newly produced resistance sources, if the change is genetic, can be immediately used for direct improvement of the original donor cultivars or used as germplasm which can be incorporated into the plant breeding program. Eighteen of the somaclonal lines from Lemont received higher disease ratings than the Lemont control rows (Table 18). Lines improved through somaculture should be tested to see that resistance to major diseases has not been reduced.

CONCLUSIONS

Somaclonal variation in rice was studied in detail through extensive evaluation for variation in morphological and agronomic characters, yielding potential, and disease resistance. A great deal of variation was observed among individuals within the somaclonal populations derived from the rice cultivars Labelle and Lemont. Most of the somaclonal lines showed variation in at least one characteristic. Variation was observed in both quantitative and qualitative characters. Many characters, including seed size, fertility, plant height, and days to heading, appeared to be controlled by a single nuclear gene. An individual plant could express more than one mutant character and a mutant character could be shared by a number of individuals within the population from a single somaclone. Some somaclonal progenies performed better when evaluated for desirable agronomic characters, such as, yield potential and disease resistance, when compared to their parent

cultivars. Further testing will be necessary to clarify the genetic base for these changes.

Variability has been generated through plant tissue culture with relatively high frequency. Edallo et al. (1981) analyzed somaclonal variation among the progeny of plants regenerated from immature embryos in maize. They demonstrated that on the average, each regenerated plant had one mutation. Oono (1984) reported the frequency of gene mutation occurring in rice somaculture was about 72% based on five characters analyzed. The present studies showed a high frequency of mutation through rice somaculture. In general, mutations occurring in cells under in vivo conditions may give cells less competitive in growth rate than surrounding normal cells. This could result in the disappearance of mutant cells during development of the plant. Thus, the occurrence and identification of mutants would be much reduced. When normal cells are grown in vitro, they are exposed to an environment where the normal developmental pathways may be altered and inhibition or limitation of growth in normal cells could result. Normal cells may be less competitive in vitro than are mutant cells. In addition, if the mutant cells have the ability to regenerate plants, the mutant can be isolated. Somaculture creates variation at a high frequency and this variation may be useful for genetic studies and plant breeding programs.

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Table 1. Frequencies of observed variation in sterility and awn production in R_0 somaclones derived from the rice cultivars Labelle and Lemont.

	Labelle	Lemont
No. of somaclones	563	235
No. of sterile somaclones	39	12
Frequency of sterility (%)	6.9	5.1
No. of awned somaclones	17	5
Frequency of awning (%)	3.0	2.1

Table 2. Comparisons of leaf blade angle, flag leaf angle, and culm angle between somaclonal lines and the rice cultivar Labelle or Lemont from which they were derived.

	Leaf blade angle			Flag leaf angle				Culm angle		
	30°	45°	90°	30°	45°	90°	90°	30°	45°	60°
Labelle		*				*			*	
Soma. lines	1	32	17			34	12	8	36	4
Lemont		*			*				*	
Soma. lines	3	37	10	1	14	33	2	6	41	3

* Cultivar characteristic

Table 3. Segregation of albino plants among two R_1 somaclonal lines from Labelle rice.

Line no.	No. of plants	Segregation for plant type		Expected ratio	X^2	p
		green	albino			
114	29	17	12	3:1	3.207	0.05-0.10
				9:7	0.068	0.75-0.90
269	36	27	9	3:1	ER = OR[+]	

[+] The expected ratio equaled the observed ratio.

Table 4. Segregation of plant height in somaclonal line 417, psuedo-blast in line 429, and small grain in line 656 in the R_1 generation.

Lines	Segregation in plant height		Segregation for pseudo-blast		Segregation for small grains	
	normal	dwarf	green	purple	normal	small
417	27	13				
429			30	10		
656					19	3
Ratio	3:1		3:1		15:1	
X^2	1.026		0.0		0.280	
p	0.25-0.50		1.0		0.50-0.75	

Table 5. Segregation of leaf tip browning among R_1 somaclonal lines from Lemont rice.

Line no.	No. of plants	Condition of leaf tips		Ratio	X^2	p
		green	brown			
258	42	38	4	15:1	0.522	0.25-0.50
304	36	26	10	3:1	0.138	0.50-0.75

Table 6. Comparisons of panicle type, panicle exsertion, culm strength, and senescence among somaclonal lines and their parent cultivar, Lebelle.

Trait	Grade	Number of somaclonal lines	Cultivar performance
Panicle type			
	Compact	2	
	Intermediate	41	*
	Open	5	
Panicle exsertion			
	Well	4	
	Moderate	38	*
	Poor	6	
Culm strength			
	High	2	
	Moderate	3	
	Intermediate	6	
	Weak	27	*
	Very weak	12	
Senescence			
	Late	1	
	Intermediate	42	*
	Early	4	

Table 7. Segregation of sterility among R_1 somaclonal lines from the rice cultivar Labelle.

Line no.	No. of plants	Fertility		Ratio	X^2	P
		fertile	sterile			
35	41	28	13	3:1	0.852	0.25-0.50
770	25	16	9	3:1	1.313	0.25-0.50
771	45	31	14	3:1	0.784	0.25-0.50
802	45	37	8	3:1	1.606	0.10-0.25
807	45	34	11	3:1	0.008	0.90
808	45	38	7	3:1	1.786	0.10-0.25

Table 8. Comparison of variability of somaclonal lines with the parent rice cultivar Labelle for six quantitatively measured characters.

Character	Somaclonal population					Labelle				
	No. of plants	Mean	STD+	Minimum	Maximum	No. of plants	Mean	STD+	Minimum	Maximum
Tiller no.	1,810	22.7	6.9	2	57	243	22.2	6.6	4	42
Seedling height (cm)	1,763	76.5	7.0	35.6	93.2	244	75.9	5.3	51.1	87.1
Height at maturity (cm)	1,814	126.3	8.6	70	158	246	126.0	6.9	110	142
Days to heading	1,764	79.1	3.4	71	109	234	78.6	2.8	74	95
Leaf length (cm)	1,335	50.7	6.6	27	73	160	50.5	6.1	36	63
Leaf width (cm)	1,336	1.6	0.2	1.0	2.2	160	1.6	0.12	1.2	1.9

+ Standard deviation

Table 9. Comparison of variability of somaclonal lines with the parent rice cultivar Lemont, in seven quantitatively measured traits.

Traits	Somaclonal population					Lemont				
	No. of plants	Mean	STD+	Minimum	Maximum	No. of plants	Mean	STD+	Minimum	Maximum
Seedling height (cm)	1,908	62.1	6.2	24	89	254	61.1	5.3	32	72
Day to heading	1,901	88.4	4.4	71	117	251	88.1	3.2	83	101
Panicle weight (g)	1,759	88.8	26.9	8.4	214.8	206	95.6	23.7	23.8	173.5
Panicle length (cm)	1,729	25.1	1.2	16.2	29.9	207	24.3	0.8	22.6	25.9
Panicle number	1,718	18.4	5.0	3	36	207	19.0	4.4	5	31
Yield per line (g)	1,781	76.1	22.8	5.1	175.	228	82.2	22.3	13.1	156.1
100-seed weight (g)	1,747	2.3	0.16	1.3	2.8	220	2.5	0.09	2.1	2.7

+ Standard deviation

Table 10. Comparison of somaclonal lines with their parent cultivars Labelle or Lemont for 10 quantitatively measured traits.

Traits measured	Range		Mean Parental values	Mean line values		
	Maximum	Minimum		Significantly higher+	Equal to cultivar	Significantly lower+
			Labelle			
Seedling height (cm)	88.0	60.6	74.3	46	90	64
Plant height (cm)	137.2	108.9	127.0	55	90	65
Tiller number	29.7	15.3	20.8	23	62	15
Days to heading	88.6	75.0	78.5	77	97	26
Leaf length (cm)	57.6	43.8	50.5	9	35	6
Leaf width (cm)	1.96	1.38	1.59	10	28	12
			Lemont			
Seedling height (cm)	72.1	52.2	60.1	38	48	14
Days to heading (cm)	95.8	80.9	88.0	10	79	11
Panicle weight (g)	117.3	63.9	98.4	6	57	39
Panicle length (cm)	27.8	23.4	24.5	81	17	2
Panicle number	22.2	10.0	19.2	13	53	35
100-seed weight (g)	2.52	2.10	2.52	0	5	95
Yield by line (g)	93.6	55.0	83.6	2	53	45

+ P=0.05

Table 11. Covariations in seedling height and plant height among Lemont somaclonal lines with seedling height either higher or lower than the control.

Lines with higher values				Lines with lower values			
Line no.	Seedling height (cm)	Line no.	Mature plant height (cm)	Line no.	Seedling height (cm)	Line no.	Mature plant height (cm)
271	88.0	271	136.7	212	73.8	200	129.0
251	85.6	226	136.4	164	73.6	102	127.6
114	85.1	251	134.2	311	73.5	118	126.9
226	84.8	287	133.6	102	73.4	288	125.9
268	83.5	106	133.1	144	73.2	311	125.2
218	82.8	222	131.9	200	73.1	212	123.4
307	82.5	307	131.8	152	72.9	205	123.3
287	81.4	114	130.4	118	72.2	152	122.9
106	81.4	188	129.7	267	71.9	267	122.7
274	79.6	218	129.6	205	71.7	164	121.0
188	79.4	268	128.7	215	65.1	144	120.0
222	78.9	274	124.7	288	64.4	215	108.9
Parent	75.9		126.0				
$LSD_{0.05}$	1.98		2.36				
r=	0.64	(Significant at P=0.01)					

Table 12. Segregation among R_1 somaclonal lines from the rice cultivar Lemont for days to heading.

Line no.	No. of plants	Days to heading		Ratio	X^2	P
		same as cultivar	longer			
155	37	30	7	3:1	0.729	0.25-0.50
299	34	25	9	3:1	0.039	0.75-0.90
304	36	25	11	3:1	0.604	0.25-0.50
693	38	24	14	3:1	2.290	0.10-0.25
850	35	28	7	3:1	0.459	0.25-0.50

+ Days to heading are the number of days from planting until 50% of the panicles have emerged from the boots.

Table 13. Covariations in panicle weight and yield among Lemont somaclonal lines with either higher or lower mean panicle weights than the Lemont control.

Lines with higher values				Lines with lower values			
Line no.	Panicle weight (g)	Line no.	Yield by line (g)	Line no.	Panicle weight (g)	Line no.	Yield by line (g)
729	117.3	726	92.6	867	84.2	435	72.0
726	108.2	404	87.6	692	83.6	749	71.6
416	101.7	416	87.2	749	83.0	692	70.6
823	100.7	426	87.1	435	82.8	867	69.1
426	100.4	823	86.4	664	79.4	429	68.8
404	99.6	729	85.6	429	77.3	664	67.5
625	98.7	806	85.2	868	76.7	417	67.2
362	98.6	363	84.7	417	76.6	868	65.7
363	98.3	362	82.4	660	76.5	656	63.4
806	97.0	625	81.7	656	73.2	660	55.7

Parent mean	88.8 (panicle wt.)	82.4 (yield)
$LSD_{0.05}$	8.8	7.4
r =	0.86 (Significant at P=0.01)	

Table 14. Frequency of variation in quantitatively measured or visually evaluated characters among 50 R_1 somaclonal lines from the rice cultivar Lemont.

No. of characters	Quantitative characters*		Visualized characters**	
	Lines	Frequency (%)	Lines	Frequency (%)
1			20	40
2	5	10	7	14
3	10	20	2	4
4	19	38	1	2
5	9	18		
6	5	10		
7	2	4		
Total	50	100	30	60

* Quantative characters evaluated were seedling height, days to heading panicle weight, panicle length, panicle number, 100-seed weight, and yield by line.
** Visualized characters evaluated were leaf blade angle, flag leaf angle, culm angle, pubescence, culm strength, and senescence.

Table 15. Frequency of occurrence of mutated characters controlled by a single recessive gene among 100 R_1 somaclonal lines from Lemont rice.

Mutated character	No. of lines	%
Normal	90	90
Days to heading	3	3
Psuedo leaf blast	1	1
Brown Leaf tip	1	1
Chlorophyll deficiency	1	1
Chlorophyll deficiency + days to heading	1	1
Brown leaf tip + days to heading	1	1
Plant height + seed size	2	2

Table 16. Effects of concentration of 2,4-D in the callus induction medium on variability among somaclonal progenies from Lemont in seven quantitatively measured characters.

2,4-D (mg/l)		Seedling height (cm)	Days to heading	Panicle length (cm)	Panicle number	Panicle weight (g)	Yield by line (g)	100-seed weight (g)
0.5	Mean	60.1	88.1	25.3	18.8	81.9	76.6	2.31
	STD*	5.1	4.4	1.1	4.8	24.4	20.8	0.15
1.0	Mean	62.8	87.6	25.2	17.5	83.0	69.6	2.30
	STD	7.3	5.2	1.4	11.4	30.3	25.3	0.19
2.0	Mean	61.0	87.9	25.2	20.6	89.3	73.6	2.33
	STD	5.4	4.1	1.4	5.4	25.5	22.1	0.12
3.0	Mean	61.1	88.5	25.3	18.6	92.1	77.0	2.33
	STD	5.9	4.1	1.2	6.2	27.3	23.6	0.16
4.0	Mean	63.0	88.3	25.5	18.1	95.2	79.1	2.39
	STD	6.4	4.1	1.1	4.8	26.2	20.7	0.14
5.0	Mean	59.9	89.2	25.3	18.4	90.9	75.6	2.38
	STD	5.9	4.6	3.4	9.4	26.0	22.8	0.15
F-test		32.2**	5.1**	4.24**	7.7**	12.0**	11.3**	28.9**
LSD		0.8	0.6	0.15	0.7	3.5	3.0	0.02

* standard deviation

Table 17. Variability of R_1 somaclonal lines from the rice cultivars Labelle and Lemont in resistance to rice sheath blight caused by *Rhizoctonia solani* Kuhn.

Cultivar or lines	Rating 3	4	5	6	7	8	9
Labelle						10	33
Somaclonal lines	2			9	22	56	269
Lemont				9	13	5	
Somaclonal lines			2	66	94	40	18

+ Scale based on a 0 to 9 rating where 0 equals no disease and 9 equals all plants dead at maturity.

Appendix 1

LITERATURE REVIEW

I. History of Plant Tissue Culture:

The cell is a basic functional unit of all living organisms. All of the complex activities of a multicellular organism depend ultimately on the activities of individual cells. A single cell removed from an organism, if kept under the proper conditions, can retain the quality of life indefinitely, as well as grow and reproduce. If a cell is separated into its constituent parts, this quality of life is lost. The concept that the individual cells of an organism are totipotent is implicit in the statement of the cell theory, as Schwann (1839) expressed the view that each living cell of a multicellular organism would be capable of developing independently if provided with the proper external conditions (White, 1954).

The idea of culturing cells, tissues, and organs of plants in isolation under controlled laboratory conditions arose during the latter part of the nineteenth century and early in the twentieth century. The most remarkable experiments of this period were undertaken by Haberlandt (1902), who made the first attempts to cultivate isolated plant cells <u>in vitro</u> on a nutrient medium. Haberlandt's plan was to study the interrelations of cells considered as elementary organisms and of establishing true, potentially perpetual tissue cultures from the isolated cells. He was unsuccessful because while attempting to utilize photosynthetic material for nutritional

reasons, he selected green mesophyll cells that were mature and highly differentiated. The meristematic activity in these specialized cells, if any, was minimal. On the other hand, Haberlandt's unsuccessful attempts to culture isolated vegetative cells from higher plants suggested the use of cell culture as well as an elegant means of studying biological events. His research stimulated later investigators to continue trying to grow tissues and organs.

The first successful organ culture was achieved by White (1934) with the demonstration of the potentially unlimited growth of excised tomato root tips in a simple liquid medium. The root-tips maintained their morphological identity as a root with the same basic anatomy and physiology as the in vivo roots of the parent plant.

The first plant tissue cultures, in the sense of two irreducible characters of a true tissue culture, undifferentiated stage and unlimited capacity for growth, were accomplished almost simultaneously by Gautheret (1939), Nobecourt (1939), and White (1939) using explants of cambial tissues isolated from tobacco and carrot. It was demonstrated that a given culture could be unlimitedly grown with formation of undifferentiated callus by repeated subcultures on a nutritional medium supplemented with external source of sugar, thiamine, and IAA (White, 1941).

Fascinated by the apparent immortality of the cultures, the scientists devoted great effort to determining the nutritional requirements for sustained growth. The discoveries of the activity of auxins (Went and Thimann, 1937) and of the potential cell division promoting power of coconut milk (liquid endosperm of coconut) allowed growth of young embryos of Datura (Van Overbeek, 1941) and cultured

carrot and potato tissues (Caplin and Steward, 1948; Steward and Caplin, 1951, 1952). Steward's group at Cornell University made numerous contributions to tissue culture in the areas of technique, nutrition, growth pattern, and plant morphogenesis. Organization and regeneration of complete plants from a cultured mass of carrot cells clearly demonstrated the totipotency of plant cells (Steward, 1958). The phenomenon of embryogenesis in carrot cultures was discovered approximately at the same time by Steward (1958) and Reinert (1959).

Studies on the chemical regulation on organogenesis in tobacco callus was undertaken by Skoog and his colleagues at the University of Wisconsin. It was found that shoot initiation in tabacco stem segments and callus could be chemically regulated by changes in the medium (Skoog and Tsui, 1948). While searching for a cell-division factor, Miller et. al. (1955) located such a factor in degraded DNA preparations. It was isolated, identified as 6-furfurylaminopurine and given the common name kinetin. The related analogues, 6-substitutedaminopurine compounds, was then synthesized. These are generally referred to cytokinin. These substances stimulate cell division in cultured plant tissues and behaved in a physiological manner similar to kinetin. Skoog and Miller (1957) advanced the hypothesis that shoot and root initiation in cultured callus can be regulated by particular ratios of auxin and cytokinin.

Guha and Maheshwari (1964 and 1966) first showed in Datura innoxia that haploids could be produced from anther culture. By the technique developed by Nitsch (1974, 1977), it became possible to culture isolated microspores of Nicotiana and Datura, to double the chromosome number of the microspores, and to produce seeds from the diploid

plants. The use of haploid tissue culture for crop breeding, specially in rice, has been actively developed in China. A detailed discussion with regards to this subject is given by Hu (1983).

It was found that callus fragments, transferred to a liquid medium and agitated on a shaker, gave a cellular suspension that could be propagated in subculture (Muir, 1953; Muir et al., 1954). Several unique techniques were developed for the culture of single isolated cells. A paper-raft nurse technique devised by Muir (1953) allowed cultured cells to utilize secreted substances from a callus mass separated from the single cell by a layer of filter paper. Another approach was to isolate the single cell in a hanging drop in a microchamber (Torrey, 1957; Jones et al., 1960). The agar-plating method of Bergmann (1960) involved the mixture of a suspension of single cells with melted agar-medium at temperature between 30°C and 35°C and plating the cells as a thin layer in a petri dish. Cornell University scientists, under Steward's direction, made extensive use of carrot suspension cultures. It became evident that this technique offered great potential for studying many aspects of cell biology (Nickell, 1956). Using a defined medium, Vasil and Hildebrandt (1965) obtained differentiation of completely organized tobacco plants from single cells in 1965.

Another development in the early 1960s was the technique for isolation and culture of protoplasts (Cocking, 1960). In general, this method involves the enzymatic digestion of the cell wall by purified preparations of cellulase and pectinase, regeneration of a new cell wall, formation of cell colonies, and eventually development of plantlets (Takebe et al., 1971). Researchers are now using this

technique for creation of hybrids by protoplast fusion between Plant species, genera, and families, uptake of blue-green algae, bacteria, viruses, and foreign organells; transfer of chromosomes and nucleic acids into isolated protoplasts, and introduction of plasmids as carriers for genetic engineering.

Today, a great deal of information is available on applications of tissue culture to various aspects of research. Among the recent books on general techniques are those by Vasil (1984) and Evans, Sharp, Yamada, and Ammirato (1983).

II. Rice Tissue Culture:

In vitro manipulation of rice cells and tissues has been more extensively investigated than for any other cereal. This is the first cereal in which callus formation and plant regeneration from either somatic or reproductive tissues was demonstrated. Callus initiation and subsequent organogenesis has been accomplished from embryo, root, shoot, leaf, immature and mature endosperm, mesocotyl, seedlings, immature panicles, leaf sheaths, seeds, anthers, pollen, and ovary. The significant events of contributions to rice tissue culture are listed in a comprehensive review by Yamada and Loh (1983), and Oono (1984).

The first attempts for in vitro culture of rice were made by a group of scientists in Japan in the 1950's (Fujiwara and Ojima, 1955; Amemiya et. al.,1956; Nakajima and Morishima, 1958). They had little success in inducing cereal callus and in maintaining good growth. Furuhashi and Yatazawa (1964) made a break through in inducing callus

from rice stem nodes. Progress was quickly made for in vitro culture of rice. Plant regenerations from many explant sources were successful. A completely synthetic medium capable of inducing and maintaining callus growth was reported by Yamada et al. (1967). Kawata and Ishihara (1968) succeeded in regeneration of plantlets from callus culture induced from the root tips of rice plants by 2,4-D. For regeneration of plantlets, the callus had to be transferred onto a differentiation medium containing 5% sucrose, 1% casein hydrolysate, 1 mg/l IAA without 2,4-D. Nishi et al. (1968) demonstrated that callus could be obtained from the roots of rice on Linsmaier-Skoog (LS) (1965) medium containing 10^{-5} M 2,4-D. When this callus was subcultured on a similar medium without auxin and incubated in the light, it differentiated into shoots and roots, and subsequently formed whole plants. Following the early success in plant regeneration from root or seed derived callus, plants have also been regenerated from callus of diverse somatic origin. Mascarenhas et al. (1975) were able to regenerate plants from seeding mesocotyl segments by transferring the proliferating callus onto medium containing 0.54 uM NAA. Diploid plants have been regenerated from scutellar tissue and cotyledonary nodes and leaf derived calli in culture (Henke et al., 1978; Wu and Li, 1971). Bahttacharya and Sen (1980) were able to regenerate plants from leaf sheath cells in culture. Bajaj et al. (1980) regenerated plants from endosperm. Examination of chromosome number proved the regenerated plants to be triploid. Morphologies of these plants exhibited broader leaves, higher growth rates and increased tillering when compared to the diploid parents.

Although plants were recovered from callus of diverse somatic

origins, the frequencies of plantlet formation were relatively low. The problems of enhancing plant regeneration has been resolved by use of immature and meristematic tissue as explants for the establishment of the culture. Nabors et. al. (1983) examined calli derived from germinating seeds of rice and distinguished two types of calli. They disignated these as embryogenic (E) and non-embryogenic (NE) callus. E callus consisted of small isodiametric cells averaging 31 um in diameter, while NE callus is composed of larger, long tubular cells averaging 52 um in diameter and 355 um in length. These two types of callus were transferred to a regeneration medium. The E callus formed plants on average of 33 times more frequently than NE callus of equal mass. Chou et. al. (1983) studied callus induction and differentiation of various plant parts of hybrid rice. The differences in callus induction and plant regeneration were observed. It was shown that callus induction frequency varied from 20 to 80% and redifferentiation of plants varied from 40-70%. It was shown that young rice spikelets could be suitable for tissue culture with highly efficient results.

The anther culture technique has been useful for the recovery of haploids, which when doubled provide homozygosity. Niizeki and Oono (1968) conducted the first successful research in culturing rice anthers to produce haploid plants. Approximately 1-2 days prior to heading, anthers containing mature pollen grains were placed onto a nutrient medium with IAA, kinetin, 2,4-D, and adenine surfate singly or in combination. The anther turned black 3 weeks after the plating. Pale yellow callus emerged after 4-8 weeks of culture. Haploid plants were regenerated after transferring the callus into a similar medium lacking 2,4-D.

Myint and deFossard (1974) obtained induction of haploid callus from rice anthers and the regeneration of rice plants. However, the experiment showed that the induction rate was very low and varied from 0.2 to 2.2%. It was suggested that callus induction from anther explants seemed the major barrier in haploid rice culture. Once callus was induced, plants could be regenerated at high frequencies without auxins, cytokinins, or organic supplements. Zhou and Yang (1981) attempted to produce haploid rice plants from ovaries. By in vitro culture of excised and unfertilized overies on the liquid N_6 medium, supplemented with 0.125 ppm, 2-methyl-4-chlorophenoxy acetic acid (MCPA), callus induction occurred at a frequency of 4.4%, and haploid plants were obtained after the calli were transferred to fresh N_6 medium with reduced concentrations of MCPA (0.033 ppm). Kuo et. al. (1977) successfully induced callus from isolated pollen grains of anthers. By preculturing the anther on liquid medium for 3 days and pretreating at about $10^{o}C$ for 10 days, many green and albino plants were regenerated from the pollen grains. However, the differentiation frequency of pollen callus was generally low and the production of albinos among the regenerated plants was relatively high.

The technology for in vitro haploid culture of rice is very advanced in China. Yin et al. (1976) described a haploid rice breeding program by which many varieties and strains have been developed for cultivation. These varieties include Tanfeng 1, Hua Yu 1 and 2, which appear to be high yielding (7500kg/ha), resistance to bacterial blight, and with wide adaptability (Hu, 1983).

For critical studies on plant genetics and breeding, a single cell culture system would be desirable. However, frustration in plant

regeneration from cell suspension culture is a major barrier to the use of this system for crop improvement. In the early 70's, Maeda (1973) obtained cell suspensions by transferring callus to liquid medium maintained on a culture shaker. Recently, more success with rice cell suspension culture has come from Ye (1984). Single cells or 2 to 3 cell aggregates were isolated in suspension culture using a metal mesh. Rice callus was effectively induced on modified B_5 medium containing 10^{-5} M of 2,4-D. Plant regeneration was promoted on MS medium supplemented with 7% sucrose.

The isolation of rice protoplast was achieved from callus cells (Tseng et. al., 1975) and from leaf blades (Tseng and Shiao, 1976). Deka and Sen (1975) obtained viable protoplasts from mesophyll cells (50-60% yield) and from callus cells (60-70% yield). The experiments showed that the age of plants, cell turgidity, and the nature of the plant parts proved to be important factors for successful isolation and culture of mesophyll protoplasts. Plating efficiencies averaging 30% were achieved in cultures of callus and mesophyll protoplasts and differentiation of roots occurred after about 2 weeks. Rice protoplasts have also been isolated from pollen callus, growing on 18.0 uM 2,4-D and 2% CW (Peking Institute of Botany, 1975). Cell wall regeneration and subsequent cell division were reported in a medium containing 0.23 μM 2,4-D and 1.3 μM BA.

The interest in protoplast fusion techniques is related to the prospect that genetic communication could be achieved beyond crosses possible by sexual means. However, the technology of somatic hybridization for cereal plants is still in a primitive state at present. Fusion of rice protoplasts and nuclear fusion were observed,

but none of the cells underwent cell division (Harn, 1973). Recently, Niizeki et. al. (1985) induced callus formation from a somatic hybrid of rice and soybean protoplasts. The fusion of rice and soybean protoplasts was obtained by incubation of the mixture in a solution containing 50% (w/v) polyethyleneglycol 1540 (PEG) at 25°C. Identification and selection of hybrid calli were based on observation of a blackish purple pigment of flavanoids in the rice calli and a softness in soybean calli.

It has been illustrated that plant regeneration from rice tissue culture occurs via either organogenesis or embryogenesis. Histological studies of shoot organogenesis from embryo-derived callus (Tamura, 1968) revealed that a distinct pattern of active anticlinal cell division occurred in the peripheral zone. A number of indentations extended towards the inside of the callus with normal leaf primordia subsequently forming around the bottom of the indentations. Nakano and Maeda (1979), with their extensive work on examination of cyto-histological differentiation, detailed plant regeneration through morphogenesis. The first sign of the morphogenesis was detected about 3 days after calli were transferred to shoot induction medium by radial divisions in the callus. At a more advanced stage, procambial and provascular tissue could be developed with formation of trichomes and chloroplasts in the callus periphery. At this time, the outermost cell layer of the callus mass became stratified and meristematic. In the latter stage, leaf primordia initiated from the surface layer of cells.

Somatic embryogenesis had initially been limited to a restricted number of species. Only in recent years has it been shown to be a more universal way of regeneration. Formation of somatic embroids has been

reported in rice tissue culture derived from rice leaf tissue, immature embryo, mature seeds, immature panicles, and microspores. Ling et. al. (1983) obtained somatic embryogenesis and plant regeneration in an interspecific hybrid of Oryza. Immature panicles were cultured on HE medium supplemented with 2,4-D and NAA 2mg/l, kinetin 3mg/l yeast extract 1360mg/l, and casin hydrolysate 300mg/l. Achievement of various developmental stages of embroids such as globular, heart-shape, scutellum-shape, and mature embryos clearly demonstrates embryogenesis. Chen (1984) obtained somatic embryogenesis and plant regeneration from culture of young inflorescences of rice. Calli were formed on LS medium with various 2,4-D concentrations. Compact yellow calli underwent somatic embryogenesis and plant regeneration through formation of compact nodules. Histological studies revealed that development scutellum-like structures and a coleoptile-coleorhize bipolar organization.

III. Somaclonal Variation and Plant Breeding.

Development of plant tissue culture techniques has been brought about many new research areas, including development of the tissue culture system itself and application of the system to crop improvement. One of the most useful features of tissue culture in a plant breeding program is its ability to generate variation. Variation generated by the use of a tissue culture cycle has been termed somaclonal variation by Larkin and Scowcroft (1981). Substantial evidence for somaclonal variation are rapidly accumulating in both monocots and dicots and sexually as well as asexually reproducing

species. Notably, this includes agronomically important graminaceous species such as rice, maize, wheat, and oats. Summation of occurrence of somaclonal variation is presented in the reviews by Larkin and Scowcroft (1981, 1983).

Heritable somaclonal variation has also been observed in both haploid and diploid tissue culture of rice (Oono, 1978; Schaeffer, 1984; Sun et. al., 1983) Phenotypic variants among somaclonal plants regenerated from rice callus were detected as soon as the first system of plant regeneration was available. The variations were in traits such as plant stature and flag leaf length.

Oono (1978) obtained more extensive results by careful mutation analysis, which exclusively demonstrated occurrence of genetic variation created through tissue culture. He examined 1121 somaclones derived from 75 calli which originated from the rice seeds of a genetically pure line (progeny of a doubled haploid from a spontaneous haploid plant).

All of these lines were analyzed for three generations in succession for five agronomic characters: chloroplast content, flowering date, plant height, morphology, and fertility. There was wide variation in seed fertility, plant height, and heading date. Only 28.1% of the plants were considered normal-parental in all of these characters, while 28% had two or more mutant characters. Segregation of the mutation in the D_2 generation was observed. Chlorophyll deficiencies were observed in the second generation of 8.4% of the lines. Sectorial analysis of plants derived from a single seed callus showed that most of the variation was induced during culture and was unlikely to pre-exist among the homozygous seeds used to initiate the

experiment. It was estimated that mutations affecting these five traits were induced in culture at a rate of 0.03-0.07/cell/division.

Sun et. al. (1983) reported genetic variability in rice somaclones for five quantitative traits, plant height, effective tiller number, grain number per panicle, heading date, and 1,000 grain weight. Analyses on 950 T_2 lines of four varieties showed that only 24.2% of the lines were normal in all traits studied. It was shown that frequency of occurrence of variation for these five traits were different with a range from 11.5% to 39.5%. It was found from comparison with the control varieties that the plant height appeared shorter, number of effective tillers increased, and the 1,000 grain weight was less. Recent data from Schaeffer (1984) on the improvement of storage proteins through rice anther culture shows the promise of gametoclonal variation for improvement of seed quality.

The prerequisite for either conventional or mutation breeding programs is to maintain large populations prior to evaluation. Using tissue culture techniques, genetic selection occurs within the laboratory and permits selection of rare mutations at frequencies of 10^{-5} to 10^{-6} because cell densities approaching 10^5 cells per ml are not uncommon. Thus, the routine use of tissue and cell culture should make it possible to screen individual variants on a scale therefore impossible. Somaculture also provides a method for handling large populations of single somatic cells from higher plants, like micro-organisms, for searching for physiological and biochemical mutants in precise way.

In higher plants, lysine as well as methionine (both essential amino acids) are derived biosynthetically from asparate. The activity

of the first enzyme appears to be regulated through feedback inhibition primarily by lysine and to a less degree by threonine. Asparate kinase activity in cell free extracts of rice shoots is also sensitive to feedback inhibition by the lysine analogue, S-(b-amino ethyl)-cysteine (SAEL). Therefore, it is possible to utilize resistance to SAEL for selection of rice mutants in which lysine biosynthesis is released from regulatory control. Schaeffer and Sharpe (1981) have been able to increase total protein content in rice by treating another cultures with SAEL. The variant individuals retained resistance to SAEL after 30 subcultures without the analog. After selfing, the F_2 generation contained individuals with a 10% increase in free lysine and a 48% increase in protein content.

Salt tolerant varieties of rice may solve the growing problem of salt in the agricultural environment, specially in Southern Asia. Thus, one of the first efforts of the IRRI tissue culture breeding program was to screen for salt-resistant varieties (Anonymous, 1981). Through somaculture, 2000 plants have been regenerated from salt resistant calli which were selected on a medium with high salt content. These regenerates were transferred to salt-water culture. The survivers have produced seeds, and their progeny are being tested in the field. Heyser and Nabors (1982) reported the development of salt and drought tolerant cell lines of rice through long-term embryogenic culture.

Oono (1977) selected diploid rice culture for tolerance to NaCl. Tolerance was retained in some regenerants and was heritable to D_3. Some callus lines resistant against 1% NaCl were also isolated after 6-12 months of subcultures of Norin 8 calli and regenerated plants.

From three resistant callus clones, 72 D_1 plants were regenerated and 19 D_2 plants from lines with normal seed fertility were examined with respect to their germination in a 1% NaCl medium. Three out of nine D_2 lines regenerated from a resistant callus clone reproduced resistant D_2 plants with segregation. Furthermore, this seedling resistance was inherited in the D_3 generation.

Plant diseases continue to be one of the major limitations to realizing yield potential in most crops. For many diseases, newly acquired genetic resistance in the host can often be short lived. The evolutionary opportunism of the pathogen often renders the host resistance ineffective after several life cycles. The most efficient way of controlling plant disease is through the identification and utilization of resistant varieties. Natural sources of resistance lie in the genetic diversity contained in existing varieties, and wild species. However, when natural sources of resistance can not be identified or depletion of genetic diversity in species is accelerated, the possibility of using artificially induced mutants must be explored. The possibility that cell culture would provide a valuable adjunct to disease-resistance breeding program has been recently reviewed by Brettell and Ingram (1979).

The advantage of *in vitro* culture systems over conventional breeding procedures for obtaining resistant mutant plants lies in the fact that a large number of individuals can be screened for a resistance character in a rapid, space-saving, and controlled way. Successful selection of plants resistant to diseases caused by pathogenic micro-organisms has been reported by Carlson (1973), who obtained spontaneous resistances of *Nicotiana tabacum* to *Pseudomonas*

tabaci and methionine sulfoximine, and by Brettell et. al. (1980), who obtained simultaneous resistance of Zea mays to Helminthosporium maydis and race T toxin.

Screening disease resistance following direct infection of plant tissue culture with pathogenic micro-organism has been reported in Tobacco (Helgeson, et. al., 1976). However, this type of selection is only restricted to systems where resistance is expressed by cultured cells. It may not be feasible to use a pathogen as a selection agent if the microorganism overgrows plant cells and the tissue culture medium, or traits being selected are not expressed by cultured cells. Ishii (1978) reported such a situation in which he tried to use aerial hyphae of Pyricularia oryza Cat. on callus tissue induced from embryos to screen for blast resistance. However, it was concluded that blast resistant genes did not manifest themselves in the callus tissue since various rice varieties with different resistance levels to the test isolate of the pathogen did not show any differences in resistance. In testing by the smear inoculation method on plantlets at an early stage of redifferentiation from embryo-callus, no difference between resistance and susceptibility was observed in various combinations of rice varieties and P. oryza strains.

It has been observed that the frequency and spectrum of variability through somaculture may be directly under the influence of culture material and method. Under carefully controlled procedures for culture manipulation and regeneration, variability among regenerated plants can be genotype-dependent. Liu and Chen (1976) tested eight sugarcane cultivars and found that among a total of 4600 plants examined, many of the plants differed from their donors. For line

F146, the frequency of morphological changes was 1.8%, while for line F156 the frequency was 34.0%.

Sun et.al.(1983), in their studies on somaclonal genetics of rice also showed that the ability to generate multiploids occurring in somaclones is different between rice varieties. The percentage of observed multiploids ranged from 0-13.3 in the indica varieties, while no multiploids were found in japonica varieties.

Miah (1985) studied the genetics of the ability to form callus in anther culture of rice. By comparing culturability of two japonica and two indica parental types, as well as, 12 F_1 hybrids from these parents a significant difference in callus induction from anthers was found. Genetic analysis showed that culturability was inherited as recessive character conditioned by a single block of genes.

In a system described by Deambrogio and Dale (1980), the effect of 2,4-D concentration on genetic variation in plants regenerated from culture was noticed. Genetic variation was detected by examining the progeny of regenerated plants. Variation occurred only at 18 uM 2,4-D for such traits as albinos, leaf shape, and fertility.

Possible causes of somaclonal variation have been discussed relatively extensively by Larkin and Scowcroft (1981), Chaleff (1981), and Shepard (1981). Virtually any type of genetic variation might contribute to the basis for somaclonal variation. Peloquin (1981) suggests that chromosome substitutions may be responsible for the changes observed in plants regenerated from tissue cultures. It is abundantly clear from the detailed meiotic analyses of somaclones by Ogihara (1981) and McCoy et al. (1982) that cell culture can result in chromosome deletions, reciprocal and non-reciprocal translocations,

inversions, and other minor rearrangements. In rice, haploid, diploid, triploid, tetraploid, and pentaploid plants have been regenerated from culture of anther and ovary calli (Nishi and Mitsuoka, 1969; Niizeki and Oono, 1971). Doubtless, similar genetic modifications must be occurring at the fine structural level, that is, single gene changes occur (Edallo et al., 1981; Barbier and Dulieu, 1980) while still others implicate cytoplasmic alterations (Sibi, 1976; Brettell et al., 1980). Larkin and Scowcroft (1981) proposed the possible origins of variation into seven categories: (1) karyotypic changes, (2) cryptic chromosome rearrangements, (3) transposable elements, (4) somatic gene rearrangements (5) gene amplification and depletion, (6) somatic gene crossing over and sister chromatid exchange, and (7) cryptic virus elimination.

It has been reported that the genetic alterations may be tissue culture induced or may have originated within the original explants. Recent evidence suggests that most of the variations arise during cell culture. In the analysis of somaclonal variation in tobacco using the sulphur locus to monitor genetic changes, Lorz and Scowcroft (1983) concluded that at least 75% of the variation among regenerants arose during the cell culture phase. Thomas et al. (1982) regenerated a number of plants from callus derived from a single potato protoplast. Morphological differences between these plants confirm their tissue culture origin. McCoy et al. (1982) show there is an increase in variants with duration in culture of oats. Oono (1978) reports that diploid regenerants from rice microspore culture show mutations which proved sometimes to be homozygous and sometimes heterozygous. If all the variations preexisted in the microspores, all the mutant diploid

regenerants would be homozygous. The heterozygous mutants were clearly derived from mutational events following the spontaneous chromosome doubling during the culture phase.

Yamada and Loh (1983) have compared a conventional mutation breeding program with a tissue culture breeding program. The introduction and manipulation of genetic variation are the principal means of plant breeders to realize their objectives. In conventional mutation breeding, ionizing radiation and chemical mutagens cause point mutations at random in the seed. Since the apical meristem is multicellular, a chimeric plant with mutated sectors will grow from the mutagenized seed (Broertjes and Keen, 1980). Somatic mutations will not be transmitted by sexual hybridyzation. Thus, desirable traits are lost unless the changes occur in a germ line cell. The significance of somaclonal variation rests on demonstrating that phenotypic variants have a genetic cause rather than resulting from an epigenetic consequence of cell culture or physiological effects. The ability for rapid clonal propagation through tissue culture makes it an ideal method for mutational research. With tissue culture techniques, somatic mutations may be saved through clonal propagation of the variant sector.

The most useful applications of somaclonal variation to crop improvement come from the creation of desirable agronomic characteristics and development of disease resistance line. High-yielding and smut-resistant lines of sugarcane developed from callus cultures are currently under test in Taiwan (Liu, 1981). A commercial cultivar of tobacco (Nicotiana tabacum) 'F211', that is more resistant to bacterial wilt than existing cultures, has been released

in Japan. The rice cultivars Hua Yu 1 and 2 developed from gametoculture in China show resistance to bacterial blight and an increase in yield.

Studies on somaclonal variation in clonal propagation of interspecific hybrids suggests a valuable potential for introgression of desirable alien genes from wide species (Cooper et. al., 1978; Nakamura et. al., 1981). Use of conventional breeding techniques for introgrssion of alien genes into crop plants are frustrated due to failure of exchange between the crop and alien genomes in the hybrids, which is probably controlled by genetic factors. However, recent successes in embryo culture of hybrids in many species suggest that such genetic factors may be inactive in cell culture, thus, making it possible to open a new way for overcoming barriers to genetic exchange.

Rice tissue culture was first initiated about 20 years ago and attention has been paid to somaclonal variation only in recent years. It is clear that efforts still need to be directed toward understanding somatic genetics and optimizing the culture system for establishment of somaclonal variation in plant breeding programs.

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Appendix 2

Media used in somaculture studies.

Constituents (mg/l)	Murashige-Skoog	Linsmaier-Skoog	Chaleff's R-2	Chu's N_6	Gamborg's B_5
NH_4NO_3	1650	1650	400	-	-
$(NH_4)_2SO_4$	-	-	-	463	134
KNO_3	1900	1900	2530	2830	2500
KH_2PO_4	170	170	170	400	-
$MgSO_4.7H_2O$	370	370	370	185	240
$CaCl_2.2H_2O$	440	440	440	166	150
$NaH_2PO_4.7H_2O$	-	-	-	-	150
$FeSO_4.7H_2O$	27.8	27.8	27.8	27.8	-
Na_2-EDTA	37.3	37.3	37.2	37.2	-
Fe-EDTA	-	-	-	-	28
$MnSO_4.H_2O$	-	-	-	-	3.1
$CuSO_4$	-	-	-	-	0.01
H_3BO_3	6.2	6.2	6.2	1.6	0.53
$MnSO_4.4H_2O$	22.3	22.3	16.8	4.4	3.1
$ZnSO_4.7H_2O$	10.6	10.6	6.62	1.5	0.45
KI	0.83	0.83	0.83	0.	1.0
$Na_2MoO_4.2H_2O$	0.25	0.25	0.25	-	0.10
$CuSO_4.5H_2O$	0.025	0.025	0.025		-
$CoCl_2.5H_2O$	0.025	0.025	0.025	-	0.006

Appendix 2 (Continued)

Media used in somaculture studies.

Constituents (mg/l)	Murashige-Skoog	Linsmaier-Skoog	Chaleff's R-2	Chu's N_6	Gamborg's B_5
Thiamine.HCL	0.5	0.5	1.0	1.0	10.0
Pyridoxine.HCL	0.5	0.5	-	0.5	1.0
Nicotinic acid	0.5	0.5	-	0.5	1.0
Inositol	100	100	100	100	100
Glycine	2.0	-	-	2.0	-
Sucrose	30,000	30,000	30,000	50,000	30,000
Agar	10,000	10,000	8,000	10,000	-
2,4-D	4.0	4.0	4.0	4.0	4.0
pH	5.8	5.8	5.8	5.8	5.5

Appendix 3

PREPARATION OF MURASHIGE AND SKOOG (MS) STOCK SOLUTIONS

The components of the Murashige & Skoog (1962) medium and stock solutions for preparation of MS medium are given in Table 1. The constituents of the MS medium are grouped into six stock solutions. These include stocks of macronutrients, micronutrients, iron, vitamins, cytokinins, and auxins.

Macronutrient stock (10x; Table 1, A). Weigh and dissolve each of the salts given in the first column in a 250 ml beaker. Transfer all of the solutions to a 1-liter volumetric flask, and add deionized water (DW) to the final volume. Store under refrigeration. Pipette 100 ml of the macromutrient stock for 1 liter of MS nutrient medium.

Micronutrient stock (100x; Table 1, B). Weigh and dissolve each of the salts given in the first column in a 140 ml beaker. Transfer to a 1-liter volumetric flask and add DW to the final volume. Store under refrigeration. Pipette 10 ml of the micronutrient stock for 1 liter of MS nutrient medium.

Iron Stock (100x; Table 1, C). Weigh and dissolve the two salts, as indicated, in a 250 ml beaker. Transfer both of the solutions to a 1-liter volumetric flask, and add DW to the final volume. Store under refrigeration. Pipette 10 ml of the iron stock for 1 liter of MS nutrient medium.

Vitamin stock (100x; Table 1, D). Weigh and dissolve each of the vitamins indicated. Transfer each of the vitamin solutions to a separate 100-ml volumetric flask and add DW to the final volume. Store

under refrigeration. Pipette 1 ml of each of the vitamin stocks for 1 liter of MS nutrient medium.

Cytokinin stock (100x; Table 1, E). Weigh 100 mg 6-Benzyladenine (BA) and dissolve it in a few drops of 1 N NaOH. Transfer the solution to a 100-ml volumetric flask. Add DW to the final volume. Store under refrigeration. Pipette 1 ml of the cytokinin stock for 1 liter of MS medium to give a final concentration of 1 mg/l.

Auxin Stock (100x; Table 1, F). Weigh 100 mg each of 2,4-dichlorophenoxyacetic acid (2,4-D) and indo-13-acetic acid (IAA). Dissolve separately in 2 ml each of 70% alcohol. Transfer each of the solutions to a 100-ml volumetric flask. Add DW to the final volume. Store under refrigeration. Pipette 1 ml of the IAA or 2,4-D stock for 1 liter of MS medium to give a final concentration of 1 mg/l.

The MS salt base is commercially available in powdered form from chemical companies; therefore, it is desirable to use the commercial salt base for the preparation of the MS medium. IAA and thiamin were prepared weekly as these chemicals degraded quickly.

Table 1. Constituents of MS medium for the rice somaculture.

Constituent	Concentration: Stock	MS medium
(A) Macronutrient	mg/1000ml (10X)	mg/1 (100ml stock gives)
NH_4NO_3	16,500	1,650
KNO_3	19,000	1,900
$CaCl_2.2H_2O$	4,400	440
$MgSO_4.7H_2O$	3,700	370
KH_2PO_4	1,700	170
(B) Micronutrients	mg/1,000 ml (100X)	mg/1 (10 ml stock gives)
$MnSO_4.4H_2O$	2,230	22.3
$ZnSO_4.4H_2O$	860	8.6
H_3BO_3	620	6.2
KI	83	0.83
$Na_2MoO_4.2H_2O$	25	0.25
$CuSO_4.5H_2O$	2.5	0.025
$CoCl_2.6H_2O$	2.5	0.025
(C) Iron	mg/1,000 ml (100X)	mg/1 (10 ml stock gives)
Na_2EDTA	3,725	37.25
$FeSO_47H_2O$	2,785	27.85

Table 1. (Continued)

(D) Vitamins	mg/100 ml (100x)	mg/l (1ml stock gives)
glycine	200	2.0
nicotinic	50	0.5
pyridoxine.HCl	50	0.5
thiamine.HCl	50	0.5
(E) Cytokinin	mg/100 ml (100x)	mg/l (ml stock gives)
BA	100	1.0
(F) Auxin	mg/100 ml (100x)	mg/l (ml stock gives)
IAA	100	1.0
2,4-D	100	1.0
		mg/l
myo-inositol		100
casein hydrolysate		500
sucrose		30,000
agar 1.0%(w/v)		10,000
pH 5.8 (Adjust to pH 5.8 with NaOH)		

STEPS IN THE PREPARATION OF THE MS MEDIUM (1-LITER VOLUME)

1. Weigh 1.0g Difco Bacto-Agar, 30.0g reagent-grade sucrose, 500mg casein hydrolysate, and 100 mg myo-inosital. Transfer them to a 1-liter flask.
2. Add approximately 600 ml DW and melt the agar by warming on a stirrer-heater.
3. Transfer 100 ml of the macronutrient stock solution to a 1-liter beaker by cylinder.
4. From each of the stock solutions previously prepared, add by pipette to the macronutrient solution: 10 ml iron, 10 ml micronutrient, and 1 ml of each vitamin (see Table 2).
5. After melting the agar, add the mixture of the stock solutions to the flask and swirl the flask for a few seconds to ensure mixing of the agar with the stock solutions. Adjust with DW to the final volume.
6. While stirring the medium use a pH meter to adjust the pH of the medium to 5.8 by adding drops of 1 N NaOH with a pasteur pipette.
7. Transfer half of the MS medium to another 1-liter flask then stopper both of the flasks with a cotton plug covered with aluminum foil. Sterilize the medium with wet heat in an autoclave. A steam pressure of 15 lb/in^2(103kPa) and a temperature of 121°C (250°F) was applied for 20 min.
8. While the medium is in the autoclave, clean the interior of the transfer chamber with a tissue soaked in 70% (v/v) ethanol. Place the sterile petri dishes (DISPO 100 x 15 mm) in the chamber to receive the autoclaved medium.
9. After the sterilized medium is removed from the autoclave, permit the hot medium to cool in the chamber for 10-15 min prior to pouring into the petri dishes. Each petri dish should receive about 30 ml of the sterilized MS medium.

Table 2. Preparation of MS media for callus induction and plant regeneration from stock solution.

Stock solution	Medium for callus induction (ml)	Medium for plant regeneration (ml)
Macronutrient (A)	100	100
Micronutrient (B)	10	10
Iron (C)	10	10
Vitamin (D)	1	1
Benzyl Adnine (E)		0.8
IAA (F)		0.5
2,4-D (F)	4	
myo-inositol	100 mg	100 mg
casin hydralysate	500 mg	500 mg
sucrose	30,000 mg	30,000 mg
agar	10,000 mg	10,000 mg
pH 5.8 (adjust to PH to 5.8 with 1N NaOH)		

Appendix 4

STEPS IN THE PREPARATION OF NODE, IMMATURE PANICLE, AND MATURE PANICLE EXPLANTS FROM RICE TILLERS

I. Sterilization:

1. Select normal healthy plants and dig them out of the soil. The morphology index of rice plants which are developing nodes, immature panicles, and mature panicles are as follows: (a) node; after the joint stages the rice stem elongates until emergence of flag leaf; (b) immature panicles; the boot stage starts with partial emergence of the flag leaf blade and lasts until the blade of the flag leaf is fully extended. When the flag leaf blade is fully extended, the last internode of the rice plant has not yet elongated; (c) mature panicles; elongation of the last internode is half completed and the rice plant is in the late boot stage. Panicles are about 4 to 6 inches long and florets have some green color.
2. Wash the rice plants thoroughly under running tap water, peel off the external leaf sheaths, and cut the rice transversely between the ultimate and penultimate nodes to a piece of boot approximately 150 mm long. Immediately place the boots in a beaker of tap water. All subsequent steps must be carried out under aseptic conditions.
3. Sterilize 1-liter flasks, jars (160 x 100 mm), and about 6 liter DW in advance by autoclaving. The flasks and jars are sealed with heavy-duty aluminum foil.

4. While the glass vials are in the autoclave, clean the interior of the transfer chamber with a tissue soaked in 70% (v/v) ethanol.
5. After the sterilized glass vials are removed from the autoclave, let the hot glass cool down in the transfer chamber. Pore 300 ml chlorox solution into a 1-liter sterilized volumetric flask. Add sterilized DW to the final volume to give a final concentration of 30 % (v/v) chlorox solution.
6. Place the boots in a sterilized jar and add the chlorox solution to the jar until they are submerged. Soak for 30 min. After sterilization the external leaf sheath appears chlorotic.
7. Following the 30 min sterilization period, pore off the chlorox solution and rinse the boots three times with sterilized DW for 20-30 sec with each rinse.

II. Inoculation:

1. Place petri dishes with MS medium containing 4 mg/l 2,4-D in the transfer chamber. Light the lamp and place the scissors and forceps in the ethanol dip.
2. Flame the forceps and pick up one sterilized boot piece from the jar at a time. Open the sheaths and pull the node, immature, or mature panicle out by peeling off the rest of the leaf sheaths with another pairs of flamed forceps. A node explant at the correct stage appears soft and yellowish with a internode about 2 cm long. The node is white.

Immature panicles should be about 1-6 cm in length, soft in texture and light yellow or light green in color. Mature panicles were green and over 10 cm long. Cuticle was well formed on the surface of the florets.

3. Flame the scissors and cut off the top 1-2 cm of the immature panicle. Cut the rest of the immature panicle into pieces about 5 mm in size and place them on the MS medium in plates. Seal the petri dish with parafilm (American Can Company).
4. Repeat the steps from 1 to 3 until the last panicle or node is plated. It is important to permit flamed instruments to cool briefly before bringing them into contact with living plant tissue.

III. Callus induction and plant regeneration:

1. Transfer the sealed petri dishes containing explants to an incubator or a culture room adjusted to 28^{o}C without fluorescent lighting.
2. Callus usually forms after several days in culture. Subculture the callus about 3 weeks after plating of explants.
3. Subdivided the callus into fragments and transfer to fresh MS medium containing 0.8 mg/l BA and 0.5 mg/l IAA for plant regeneration. Each petri dish should contain five calli, each one about 0.5-1.0 cm in diameter. Transfer instruments must be flamed and sterile technique used throughout the subculture period.
4. Incubate the cultures in a growth chamber or culture room at

28°C with a 16-hr light period. Shoot formation occurs after about 4 weeks and growth of plantlets was facilitated by transferring shoots with subtending callus to fresh regeneration medium. It may be necessary to prepare subcultures of the callus every 3-4 weeks during the course of the experiments.

Appendix 5

STEPS FOR PREPARATION OF ROOT AND MESOCOTYL EXPLANTS

1. Weigh out 19.5 gram Difco potato dextrose agar (PDA) and place it in a 1-liter flask. Add 500 ml DW to the flask.
2. Sterilize the PDA medium, 1 liter DW, and several 140-ml beakers.
3. Dehull 100 rice seeds and prepare a 30% chlorox solution (same as step 5 in appendix II). Add approximately 50 ml of the prepared chlorox solution to a 140 ml sterilized beaker.
4. Drop the dehulled seeds in the chlorox solution and soak for 30 minutes.
5. Following the sterilization, pour off the chlorox solution and rinse three times with sterilized DW for 20-30 sec each rinse.
6. Place about 8 to 10 sterilized seeds in each petri dish containing PDA with flamed forceps. Seal the petri dish with parafilm.
7. Incubate the seeds in a growth chamber at 28 oC for germination. Root and shoot formation occurs about 3 to 5 days later.
8. Germinating seeds may or may not be contaminate with microorganisms. Use only the uncontaminated seedlings.
9. Flame a pair of scissors and cut root tip (about 1 cm) or shoot (about 1 cm from the point of attachment of the root) into pieces about 2 to 5 mm in length and transfer them to MS medium containing 2 mg/l 2,4-D.
10. Incubate in the growth chamber at 25-28^{o}C without light. Callus formation occurs after about 7 days for root explants and after 15 to 20 days from shoot explants.

11. For production of cell suspension, transfer the induced callus to liquid MS medium containing 0.1 mg/l 2,4-D and incubate on a shaker, set at 100 RPM. Incubate the cell suspension for about 7 to 10 days.
12. For callus proliferation, transfer callus to fresh solidified MS medium containing 2 mg/l 2,4-D every 3 weeks.

Appendix 6

STEPS FOR PREPARATION OF EMBRYO OR ENDOSPERM EXPLANTS

1. Repeat steps 3-5 in Appendix 5 for sterilization of rice seeds.
2. Place sterilized seeds in a sterile petri dish containing sterilized DW for 24 hr.
3. Cut soaked rice seeds into two parts along the scutellum. One side has the embryo explant which is placed onto MS medium containing 2 mg/l 2,4-D. The other portion of the seed has the endosperm explant which is cut into 2 or 3 pieces (1-2 mm) and placed onto the same medium.
4. Repeat steps 10-12 in Appendix 5 for callus induction.

Appendix 7

Table 1. Mean values of quantitatively measured characters among somaclonal lines and their parent cultivar Labelle.

Line no.	Tiller number	Seedling height [a] (cm)	Plant height [b] (cm)	Days to heading	Leaf length [c] (cm)	Leaf width [c] (cm)
Labelle	19.3	74.6	124.4	79.7	50.5	1.6
16	20.0	71.9	117.0	83.4	49.3	1.5
17	18.8	78.0	127.4	78.0	52.4	1.6
18	22.3	74.0	116.6	79.1	49.0	1.5
20	20.9	73.2	125.1	78.6	48.7	1.4
21	19.1	73.4	123.1	78.1	46.2	1.6
22	18.2	80.6	106.5	78.5	51.5	1.5
26	19.1	73.2	125.7	80.4	54.1	1.6
27	18.6	63.2	97.8	88.6	43.8	2.0
28	19.5	73.8	123.6	78.0	47.9	1.6
29	20.4	76.1	126.7	80.1	50.2	1.7
30	22.1	78.3	128.5	80.7	53.6	1.5
31	20.3	77.8	127.6	81.5	50.4	1.6
32	19.9	79.4	126.2	77.0	53.6	1.5
33	17.5	75.0	124.6	79.6	54.2	1.6
34	19.6	70.0	120.8	80.8	49.5	1.7
35	14.7	77.0	116.0	85.8	53.6	1.6
36	18.5	76.5	129.1	79.2	52.6	1.7
37	22.8	74.9	123.0	82.3	48.4	1.5
38	17.7	76.9	124.4	79.6	46.9	1.5
39	21.4	72.2	128.9	80.3	48.5	1.6
42	23.4	66.8	117.4	80.6	51.6	1.5
43	23.4	71.5	117.8	78.4	46.9	1.5
44	19.8	69.6	118.9	78.7	48.1	1.6
49	17.2	73.8	124.2	79.9	49.6	1.7
50	15.8	82.1	130.1	79.6	56.4	1.6
52	19.8	72.1	117.1	80.4	50.6	1.6
53	14.7	78.9	123.9	82.2	53.6	1.7
54	15.3	73.2	122.0	83.1	49.2	1.6
55	21.0	71.6	124.0	81.3	52.6	1.5
58	21.9	73.7	121.4	79.9	52.6	1.6
59	15.9	83.3	132.2	80.5	57.6	1.6
60	16.8	78.4	127.0	79.6	50.8	1.6
61	21.9	71.7	122.9	79.4	49.6	1.6
62	18.4	73.2	116.3	79.9	51.5	1.6
63	18.6	73.9	126.5	79.4	54.6	1.6
65	21.4	73.9	124.0	81.0	47.9	1.6
66	21.5	72.4	120.2	78.2	49.3	1.6
70	20.2	73.4	128.4	81.3	51.9	1.7
71	17.6	70.4	119.8	79.6	49.9	1.5
72	23.5	73.6	129.1	79.4	49.7	1.6
73	24.1	70.9	117.5	79.3	46.4	1.4

Table 1. (continued)

Line no.	Tiller number	Seedling height [a] (cm)	Plant height [b] (cm)	Days to heading	Leaf length [c] (cm)	Leaf width [c] (cm)
80	21.4	77.3	125.7	79.4	51.0	1.6
81	19.0	75.9	125.7	80.7	50.9	1.6
83	16.6	73.7	128.4	81.2	52.2	1.7
85	26.3	71.6	120.8	80.7	51.5	1.5
88	21.0	66.7	122.6	79.3	48.9	1.6
89	19.7	77.3	124.4	81.9	52.2	1.7
95	24.6	75.3	124.2	78.3	47.0	1.5
98	17.8	71.0	123.9	78.0	49.8	1.7

[a] At maximum tillering stage (8 weeks after seeding)

[b] At maturity

[c] Penultimate leaf

Table 2. Mean values of quantitatively measured characters among somaclonal lines and their parent cultivar Labelle.

Line no.	Tiller number	Seedling height [a] (cm)	Plant height [b] (cm)	Days to heading
Labelle	22.2	75.9	126.0	78.6
102	22.2	73.5	127.6	78.9
103	24.0	77.5	131.4	78.5
106	21.7	81.4	133.1	80.3
114	21.9	85.1	130.4	77.7
118	24.7	72.2	126.9	78.8
119	22.3	76.1	126.4	80.4
133	19.2	75.8	126.1	82.2
144	24.8	73.2	120.0	79.6
152	19.4	73.0	123.0	81.2
156	25.9	74.5	124.7	78.9
164	29.7	73.6	121.0	78.5
178	24.4	75.8	127.3	80.6
179	19.4	76.3	133.5	77.0
188	23.5	80.0	129.7	81.6
198	26.4	76.5	132.0	78.6
199	22.5	77.6	124.3	80.5
200	23.3	73.1	129.2	78.7
201	22.3	75.4	126.8	79.7
205	22.2	71.7	123.3	77.4
206	20.5	74.2	118.5	81.9
207	--	77.5	--	78.4
209	22.4	74.2	119.5	79.0
212	24.2	73.8	123.4	79.3
215	25.5	65.1	108.9	78.6
218	23.0	82.9	129.6	78.6
221	25.1	75.9	122.0	76.2
222	23.6	78.9	131.9	75.7
223	21.2	77.8	123.7	79.8
226	24.6	84.8	136.4	78.0
242	23.3	75.4	122.7	79.0
245	20.6	74.9	128.0	81.6
248	15.5	75.0	117.2	78.5
249	19.9	74.9	127.7	80.5
251	20.1	85.7	134.2	77.2
253	22.6	75.6	127.1	77.3
260	24.4	76.3	123.9	78.0
267	17.5	71.9	122.1	79.6
268	19.8	83.5	128.7	80.1
269	20.8	76.7	130.1	77.6
270	25.2	78.4	126.6	79.2
271	22.6	88.0	136.7	78.7
274	21.7	79.6	124.9	79.4

Table 2. (continued)

Line no.	Tiller number	Seedling height [a] (cm)	Plant height [b] (cm)	Days to heading
281	22.8	74.9	122.0	79.3
287	23.2	81.4	133.7	78.3
288	26.0	64.4	125.9	78.6
294	25.3	74.3	118.7	79.2
298	21.9	77.5	125.9	77.8
299	--	74.6	--	79.0
305	17.7	77.9	124.9	80.6
307	23.4	82.5	131.8	78.9
308	24.0	78.0	126.5	80.4
311	25.7	73.5	125.2	76.0

[a] At maximum tillering stage (8 weeks after seeding)

[b] At maturity

Table 3. Mean values of quantitatively measured characters among somaclonal lines and their parent cultivar Labelle.

Line no.	Seedling height [a] (cm)	Plant height [b] (cm)	Days to heading
Labelle	72.1	129.0	78.3
279	72.2	117.0	77.3
312	69.5	123.6	78.5
313	71.9	126.0	78.7
317	75.6	128.8	78.3
323	69.6	125.3	77.0
324	67.1	124.2	- -
329	74.5	128.4	78.3
331	68.4	123.9	77.8
338	70.0	121.0	80.5
339	69.4	127.6	80.7
340	73.9	125.8	78.7
345	75.5	133.0	81.3
352	74.2	124.6	77.0
360	70.9	128.4	77.9
366	70.7	131.0	80.8
369	- -	- - -	79.6
371	69.6	124.2	79.3
372	71.5	129.7	79.9
373	71.9	128.6	79.2
376	69.3	124.0	78.8
378	69.2	123.8	79.5
383	72.8	127.1	79.7
393	72.0	131.9	77.9
398	73.0	128.7	77.9
401	76.2	135.2	81.1
406	76.6	128.4	78.1
408	71.5	126.7	79.6
414	72.9	125.8	77.7
428	69.1	123.8	78.3
433	70.5	127.7	80.9
434	70.6	129.0	76.8
437	74.0	128.8	77.6
642	78.6	119.4	76.7
676	75.9	129.2	79.3
677	73.5	126.1	79.9
679	74.6	130.2	80.0
680	72.2	128.7	80.7
685	77.0	130.2	78.7
689	80.0	132.2	76.9

Table 3. (continued)

Line no.	Seedling height [a] (cm)	Plant height [b] (cm)	Days to heading
690	78.3	132.7	78.8
699	72.8	128.4	80.6
703	73.3	131.7	77.1
710	70.4	125.7	80.0
712	66.7	126.4	79.2
713	72.0	127.7	78.0
715	72.7	129.1	79.6
718	68.6	127.5	81.2
720	70.4	128.4	78.9

[a] At maximum tillering stage (8 weeks after seeding)

[b] At maturity

Table 4. Mean values of quantitatively measured characters among somaclonal lines and their parent cultivar Labelle.

Line no.	Seedling height [a] (cm)	Plant height [b] (cm)	Days to heading
Labelle	74.4	128.7	77.5
721	77.1	130.8	76.5
730	72.3	122.3	75.0
731	70.3	128.4	78.8
732	71.7	127.5	80.0
734	67.9	117.7	77.6
735	66.2	117.1	78.2
736	72.7	127.6	76.8
740	78.9	133.3	79.3
741	60.0	127.9	79.5
743	70.8	130.2	81.0
751	70.3	124.2	77.9
752	72.4	128.7	78.7
753	73.7	128.8	79.4
765	78.4	135.7	79.3
766	68.4	128.6	77.5
769	74.6	133.3	80.3
770	71.3	131.5	77.6
771	66.0	126.9	78.6
773	73.5	125.4	76.9
774	65.9	117.7	79.4
778	72.6	125.3	79.8
779	68.7	124.5	75.8
780	68.2	128.8	76.6
781	74.0	128.2	79.2
785	79.4	131.7	78.7
790	78.4	133.6	77.5
791	78.0	131.6	79.5
796	72.0	124.3	80.2
802	72.1	122.8	82.8
807	75.9	129.3	81.3
808	71.3	128.8	81.8
809	74.0	127.8	78.5
811	70.7	123.6	77.3
813	75.6	127.8	79.3
814	72.0	125.5	80.5
815	74.3	132.0	80.0
817	74.3	124.2	78.8
824	62.4	125.0	81.2
842	72.9	123.8	79.2
843	75.1	133.5	79.5

Table 4. (continued)

Line no.	Seedling height [a] (cm)	Plant height [b] (cm)	Days to heading
844	73.3	130.3	77.8
856	67.7	126.1	78.9
857	64.4	125.6	82.3
860	68.7	124.1	78.4
865	73.2	127.1	77.2
866	74.1	124.3	76.6
872	73.7	137.2	80.2
878	74.6	133.7	80.6
983	72.2	131.2	81.6

[a] At maximum tillering stage (8 weeks after seeding)

[b] At maturity

Table 5. Mean values of quantitatively measured characters among R_1 somaclonal lines and their parent Lemont.

Line no.	Seedling height [a/] (cm)	Days to heading	Panicle Length (g)	Panicle number	Panicle weight (g)	Yield per. line (g)	100-seed weight (g)
Lemont	59.0	87.9	24.6	19.3	101.3	84.9	2.54
10	59.2	91.6	25.1	18.9	89.5	73.7	2.22
24	59.3	90.1	25.3	16.7	96.5	79.1	2.12
25	57.0	90.1	25.2	16.2	88.1	72.9	2.13
51	55.7	90.0	25.7	20.9	105.7	88.8	2.18
64	60.0	88.8	25.5	18.3	90.7	70.3	2.46
67	61.7	89.2	26.6	20.5	112.4	91.7	2.27
82	59.3	88.1	25.2	18.6	82.6	67.0	2.24
84	71.9	86.7	26.5	17.1	90.7	66.3	2.10
97	61.5	88.0	24.6	15.0	78.4	58.6	2.23
101	59.5	87.9	25.2	13.6	78.8	64.7	2.50
104	58.9	87.7	24.9	14.2	88.8	72.9	2.50
105	58.1	88.4	26.3	17.8	97.1	79.3	2.48
113	65.2	88.6	26.0	17.5	104.6	83.3	2.37
120	61.6	87.4	25.1	17.4	95.8	77.7	2.43
121	60.4	86.1	25.2	17.5	94.1	77.6	2.31
155	52.2	91.5	24.9	16.9	91.4	72.7	2.36
169	66.5	87.7	26.0	12.5	72.1	55.0	2.10
180	58.6	88.0	25.6	17.5	95.0	76.8	2.40
183	57.8	88.1	25.7	17.7	87.1	74.1	2.41
184	60.0	89.6	25.2	19.1	97.6	80.5	2.36
185	61.0	86.3	26.0	18.3	95.8	79.8	2.48
186	59.5	87.4	25.6	18.9	99.4	81.7	2.43
196	63.5	86.5	26.3	19.0	112.7	93.6	2.47
203	59.1	87.4	25.2	18.6	98.1	81.1	2.46
204	60.9	87.0	25.6	18.3	101.2	82.2	2.47
207	56.7	88.6	25.3	18.2	100.9	86.5	2.46
214	57.7	88.4	25.4	19.2	83.2	66.1	2.28
217	56.8	87.3	25.5	18.1	97.4	78.7	2.39
218	63.2	89.0	25.5	17.5	93.0	73.5	2.23
219	58.0	89.8	25.4	17.8	86.1	68.8	2.35
224	58.1	86.9	26.1	20.2	92.1	71.3	2.23
231	62.4	85.3	25.3	17.4	86.6	68.2	2.27
236	57.3	85.8	25.0	16.3	80.1	64.6	2.35
258	60.0	88.9	26.0	17.0	95.4	76.5	2.15
261	61.9	84.9	25.6	17.9	99.7	80.3	2.23
262	63.4	86.4	25.9	17.9	84.1	66.5	2.32
266	63.1	85.8	25.4	17.4	91.3	73.4	2.26
267	66.8	87.2	25.9	21.0	104.6	84.6	2.35
286	60.8	86.0	24.7	18.2	93.9	77.7	2.30
289	55.1	86.5	25.2	17.8	87.5	72.0	2.33
296	58.5	88.6	25.8	18.3	90.6	77.6	2.34

Table 5. (continued)

Line no.	Seedling height [a] (cm)	Days to heading	Panicle Length (g)	Panicle number	Panicle weight (g)	Yield per line (g)	100-seed weight (g)
297	60.2	87.8	26.1	21.1	100.4	81.3	2.31
299	62.9	91.7	25.6	17.8	103.3	87.3	2.33
303	61.2	85.3	25.2	18.2	98.2	84.5	2.29
304	62.3	90.9	25.6	15.2	75.9	60.6	2.18
321	60.0	86.3	24.3	14.6	79.3	65.5	2.31
322	62.2	88.0	24.7	15.1	82.2	70.3	2.42
333	59.1	87.7	25.8	19.5	97.8	80.7	2.47
345	61.8	89.2	25.8	16.9	91.5	76.9	2.29
347	58.7	88.4	25.5	17.8	85.9	71.8	2.30

[a] At the maximum tillering stage (8 weeks after seeding)

Table 6. Mean values of quantitatively measured characters among somaclonal lines and their parent Lemont.

Line no.	Seedling height [a/] (cm)	Days to heading	Panicle Length (g)	Panicle number	Panicle weight (g)	Yield per line (g)	100-seed weight (g)
Lemont	61.1	88.1	24.3	19.0	95.6	82.4	2.51
353	62.0	88.7	25.7	19.5	90.7	76.6	2.38
362	63.0	88.2	24.4	19.2	98.6	82.4	2.44
363	63.2	87.6	25.0	18.5	98.3	83.5	2.39
374	60.3	87.5	25.0	19.1	92.7	80.0	2.37
375	60.5	88.1	25.7	17.3	88.4	76.0	2.46
376	64.6	86.6	25.1	19.0	95.1	83.5	2.43
395	62.9	88.5	26.1	22.2	90.9	76.7	2.48
404	62.3	87.6	25.5	19.2	99.6	87.6	2.20
416	63.3	87.9	26.1	18.0	101.7	87.2	2.45
417	58.3	88.9	23.8	17.0	76.7	67.2	2.29
418	58.7	88.0	24.7	16.8	88.8	77.5	2.37
426	65.1	87.6	24.8	19.6	100.4	86.4	2.40
427	65.0	88.7	25.6	21.9	95.1	82.5	2.41
429	60.9	89.8	24.1	16.8	77.3	68.8	2.23
430	62.3	90.1	24.5	17.6	85.0	76.8	2.43
435	59.9	88.4	25.0	16.1	82.8	72.0	2.23
436	61.8	89.4	25.4	17.9	90.5	78.4	2.45
473	72.1	80.9	24.0	10.1	32.4	24.9	1.82
577	61.3	87.9	25.0	16.2	88.9	76.4	2.40
582	58.6	87.7	25.2	19.1	94.3	82.0	2.34
591	57.1	89.4	24.2	19.7	89.5	75.6	2.28
625	58.7	91.1	24.2	19.6	98.7	81.7	2.27
651	57.3	88.7	26.1	21.6	85.5	73.3	2.26
656	59.7	86.7	23.4	16.0	73.2	63.4	2.23
657	61.6	89.4	25.0	21.9	90.7	78.0	2.35
660	69.7	90.6	27.8	17.2	76.6	55.7	2.00
664	62.5	90.5	26.3	18.0	79.4	67.5	2.31
666	60.0	89.8	25.7	20.0	90.9	80.9	2.27
684	63.4	88.9	24.9	17.7	85.8	82.0	2.36
687	57.5	89.0	25.1	19.3	91.3	75.7	2.31
688	66.1	99.7	25.8	17.0	88.2	76.6	2.44
692	63.6	89.4	25.6	15.3	83.6	70.6	2.27
693	65.1	95.8	25.2	16.7	82.4	87.1	2.33
719	65.5	87.8	25.9	18.5	87.4	72.6	2.45
723	65.9	87.6	24.9	18.0	86.1	74.4	2.51
726	61.6	88.6	25.7	21.7	108.2	92.6	2.46
729	67.4	87.2	25.4	20.6	119.3	85.2	2.41
749	60.8	88.5	25.6	18.2	83.0	71.6	2.32
750	62.8	86.7	25.0	20.7	92.5	75.5	2.29
806	59.6	87.6	25.2	21.1	97.0	84.7	2.35
823	60.3	89.1	24.1	19.7	100.7	85.6	2.35
847	62.1	87.0	24.9	20.0	85.9	76.4	2.28

Table 6. (continued)

Line no.	Seedling height [a/] (cm)	Days to heading	Panicle Length (g)	Panicle number	Panicle weight (g)	Yield per line (g)	100-seed weight (g)
848	57.6	87.1	24.4	18.9	86.6	76.8	2.29
849	62.0	88.9	25.7	18.1	89.3	79.1	2.14
850	61.0	90.3	24.4	18.3	87.7	72.1	2.26
852	64.1	88.3	24.8	17.6	86.1	75.7	2.38
862	62.0	87.6	25.0	15.3	87.6	79.3	2.52
867	60.4	87.7	24.4	18.6	84.2	69.1	2.22
868	61.5	87.9	24.0	16.0	76.7	65.7	2.30
875	63.9	86.5	-	-	63.9	79.9	2.23

[a/] At the maximum tillering stage (8 weeks after seeding)

Appendix 8

Table 1. Visually observed characters among R_1 somaclonal lines and their parent Labelle.

	Leaf Blade Angle			Flag Leaf Angle		Culm Angle		
Line	30°	45°	90°	90°	90°	30°	45°	60°
Labelle		+		+			+	
16			+	+			+	
17		+			+		+	
18		+		+		+		
20		+		+				+
21		+		+			+	
22			+		+	+		
26		+		+			+	
27	+			+		+		
28		+		+				+
29		+		+			+	
30		+		+		+		
31		+		+		+		
32		+		+				+
33		+			+		+	
34		+		+				+
35			+	+			+	
36		+		+			+	
37		+		+			+	
38			+		+		+	
39			+		+		+	
42			+		+		+	
43		+		+			+	
44		+		+			+	
49		+		+			+	
50			+	+			+	
52		+		+			+	
53		+		+			+	
54		+		+			+	
55		+		+			+	
58			+	+			+	
59			+		+		+	
60			+	+			+	
61		+			+		+	
62		+		+		+		
63		+		+		+		
65		+		+			+	
66		+		+			+	
70		+		+			+	
71		+		+			+	
72		+		+			+	
80		+		+			+	
81			+		+		+	

Table 1 (continued)

	Leaf Blade Angle			Flag Leaf Angle		Culm Angle		
Line	30°	45°	90°	90°	90°	30°	45°	60°
83		+		+			+	
85			+		+		+	
88			+		+		+	
89		+		+		+		
95		+			+		+	
98			+		+		+	
Total	1	33	14	35	13	8	36	4

Table 2. Visually observed characters among R_1 somaclonal lines and their parent Labelle.

Line	Culm Strength				
	High	Moderately high	Intermediate	Weak	Very weak
Labelle				+	
721			+		
730				+	
731					+
732					+
734			+		
735		+			
736				+	
740				+	
741				+	
743				+	
751				+	
752					+
753				+	
765					+
766				+	
769			+		
770				+	
771				+	
773		+			
774	+				
778					+
779				+	
780					+
781	+				
785			+		
790				+	
791				+	
796		+			
797		+			
802				+	
807				+	
808				+	
809					+
811				+	
813					+
814				+	
815				+	
817				+	
824				+	
842			+		
843					+
844				+	
856				+	
857				+	

Table 2 (continued)

Line	Culm Strength				
	High	Moderately high	Inter-mediate	Weak	Very weak
860				+	
865					+
866					+
872				+	
878					+
983				+	
Total	2	4	5	27	12

Table 3. Visually observed characters among somaclonal lines and their parent Lemont.

Line	Panicle Type			Panicle Exsertion			Senescence		
	Compact	Intermediate	Open	Good	Moderate	Poor	Late	Intermediate	Early
Lemont		+			+			+	
10		+			+			+	
24		+			+			+	
25		+			+			+	
51		+				+		+	
64		+			+			+	
67		+			+			+	
82		+		+	+				+
84		+			+			+	
97		+			+			+	
101		+			+			+	
104		+			+			+	
105		+				+		+	
113		+			+				+
120		+			+			+	
121		+			+		+		
155	+				+			+	
169			+		+			+	
180		+			+			+	
183			+		+			+	
184		+			+			+	
185		+			+			+	
186		+			+			+	
196			+		+			+	
203		+			+			+	
204		+			+			+	
207		+			+			+	
214		+			+			+	

Table 3 (continued)

Line	Panicle Type			Panicle Exsertion			Senescence		
	Compact	Intermediate	Open	Good	Moderate	Poor	Late	Intermediate	Early
217	+				+			+	
218		+			+			+	
219		+			+			+	
224		+				+		+	
231		+			+			+	
236		+			+			+	
258		+			+			+	
261		+			+			+	
262		+				+		+	
266			+		+			+	
267		+			+				+
286		+			+			+	
289		+			+			+	
296			+	+				+	
297		+			+			+	
299		+			+			+	
303		+		+				+	
304		+			+			+	
321		+				+		+	
322		+		+				+	
333		+				+		+	
345		+			+			+	
347		+			+				+
Total	2	43	5	4	40	6	1	45	4

Table 4. Visually observed characters among somaclonal lines and their parent Lemont.

	Leaf Blade Angle			Flag Leaf Angle				Culm Angle		
Line	30°	45°	90°	30°	45°	90°	90°	30°	45°	60°
Lemont		+			+				+	
10		+		+				+		
24		+			+				+	
25		+			+				+	
51		+				+			+	
64		+			+					+
67		+				+			+	
82		+				+				+
84			+			+			+	
97			+			+			+	
101		+				+			+	
104		+			+				+	
105		+				+				+
113		+			+				+	
120		+			+				+	
121		+			+				+	
155			+			+			+	
169			+			+			+	
180		+				+			+	
183		+				+			+	
184		+				+			+	
185		+				+			+	
186		+				+			+	
196		+				+			+	
203		+			+				+	
204			+			+			+	
207	+				+				+	
214		+				+		+		
217	+				+				+	
218		+				+		+		
219		+				+			+	
224			+				+	+		
231			+			+			+	
236		+				+			+	
258		+				+			+	
261		+				+		+		
262		+				+			+	
266		+				+			+	
267		+				+			+	
286			+				+	+		
289	+				+				+	
296		+				+			+	
297			+			+			+	
299			+			+			+	
303		+				+			+	

Table 4 (continued)

	Leaf Blade Angle			Flag Leaf Angle				Culm Angle		
Line	30°	45°	90°	30°	45°	90°	90°	30°	45°	60°
304		+				+			+	
321		+				+			+	
322		+				+			+	
333		+				+			+	
345		+				+			+	
347		+				+			+	
Total	3	37	10	1	12	35	2	6	41	3

Appendix 9

In 1985 the lines generated from Calrose 76 were naturally infected with rice blast caused by Pyricularia oryzae L. at the tillering stage of growth. The response of each somaclonal line to rice blast was measured by harvesting a leaf from the bottom portion of randomly selected plants of each line and from ten penultimate leaves of the same plants. The number of rice blast lesions were counted and used as an estimate of blast severity.

Response to Rice Blast

Natural infection by P. oryzae among R_2 progeny lines derived from plants in the R_1 generation of Calrose 76 was evaluated during the 1985 growing season. There were apparent differences in response to rice blast between progeny lines and the parent cultivar and between R_2 progeny lines and the R_1 families (Table 1). The mean number of lesions on bottom leaves was lower in the R_1 lines numbered 37, 40, 41, 43, and 45 than for the parent cultivar. The number of leaf lesions from the progeny row of line 45 was about 16% less than the parent. The total mean number of leaf lesions from the progeny rows of the R_1 lines 40, 41, 42, and 45 remained lower than the parent cultivar, although the parent showed a greater reduction in the number of leaf lesions on the top leaves. The R_1 family line 45 showed a reduction of about 10% in the number of leaf lesions. Based on the mean lesion numbers among R_1 family lines, the response to blast could be classified into three groups: very severe, severe, and less severe infections. The number of leaf lesions from progeny rows of R_1 lines

40 and 41 was low (Table 1). In contrast, the progeny rows of the R_1 lines 47 and 48 showed high lesion numbers. Comparison of leaf lesion numbers between bottom and top leaves showed that the number of leaf lesions on the top leaves was reduced in all the lines. However, the rate of reduction in lesion number was different between the lines. The greatest reduction in leaf lesion number was in line number 38. The least reduction occurred in line number 44. The top leaf was produced after the plants were flooded. The reduction in number of leaf lesions might indicate the level of partial resistance to infection by P. oryzae. Within R_2 populations, individual R_2 lines also varied in response to P. oryzae in terms of lesion number. The difference between the line with the highest total lesion number and the line with lowest lesion number was 95 lesions over the 10 leaf sample.

Rice blast is a major disease of rice which causes severe damage and reduction in yield. It is essential to develop resistant varieties. Our primary field evaluation on progenies of regenerated plants showed apparent differences in response to infection by P. oryzae. Field or greenhouse testing of tissue culture derived progenies would be a possible way for screening somaclonal lines for changes in resistance to rice blast resistance.

Table 1. Variation in number of blast lesions, caused by Pyricularia oryzae, produced on progenies of somaclones and comparison to their parental cultivar Calrose 76.

Lines of cultivar	Bottom leaf Range		Means	S+	Penultimate leaf Range		Means	S+	Total Range		Means	S+
CR-37	20	116	62	4.5	16	67	35	3.0	48	152	100	5.1
Calrose 76			71				24				94	
CR-38	34	86	60	4.0	7	48	28	2.3	42	137	88	4.8
Calrose 76			51				22				68	
CR-39	17	107	55	4.0	12	72	36	2.9	38	159	91	5.1
Calrose 76			47				31				78	
CR-40	12	78	41	3.1	5	52	25	2.1	23	112	66	3.8
Calrose 76			45				22				67	
CR-41	17	73	40	3.1	5	51	23	2.4	25	103	63	4.1
Calrose 76			46				23				69	
CR-42	41	91	68	3.9	31	62	45	2.7	74	136	112	4.8
Calrose 76			79				42				121	
CR-43	49	84	63	3.5	30	66	47	2.8	82	145	110	4.5
Calrose 76			67				43			110		
CR-44	47	93	64	3.5	36	71	53	2.9	83	148	117	4.6
Calrose 76			58				30			89		
CR-45	41	89	68	3.3	40	69	54	2.8	79	149	121	4.6
Calrose 76			81				54			135		
CR-46	32	99	62	3.6	27	75	48	2.9	59	169	110	4.9
Calrose 76			59				45			104		
CR-47	50	105	78	3.4	40	101	63	3.3	90	201	141	5.4
Calrose 76			76				64			140		
CR-48	61	119	89	4.6	51	96	69	3.1	112	202	158	5.6
Calrose 76			87				61			148		

+ S = Standard deviation

VITA

Jun Cao was born on May 9, 1954 in Beijing, the People's Repulic of China. He received his elementary and secondary education in Beijing. His undergraduate studies were taken at Hei Long-jiang Agricultural College, Hei Long-jiang province, China, and he graduated from college with a major in Agronomy in 1978. Upon graduation, Mr. Jun was commissioned to the same college where he worked as an instructor for 6 months. In 1979, he was admitted to the Chinese Academy of Agricultural Sciences to study for the M.S. degree in agronomy. Mr. Jun satisfactorily completed the requirements and was granted a M.S. degree in Agronomy from the Chinese Academy of Agricultural Science in 1982. He entered Louisiana State University upon receiving a fellowship from the Rockfeller Foundation. At present, he is a candidate for the degree of Doctor of Philosophy with a major in Plant Pathology and a minor in Agronomy.

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